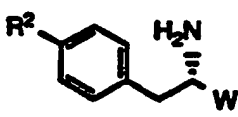




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07C 233/08	A1	(11) International Publication Number: WO 94/00420 (43) International Publication Date: 6 January 1994 (06.01.94)								
<p>(21) International Application Number: PCT/US93/05879</p> <p>(22) International Filing Date: 18 June 1993 (18.06.93)</p> <p>(30) Priority data:</p> <table border="0"> <tr> <td>07/900,959</td> <td>18 June 1992 (18.06.92)</td> <td>US</td> </tr> <tr> <td>07/995,789</td> <td>23 December 1992 (23.12.92)</td> <td>US</td> </tr> <tr> <td>08/079,239</td> <td>17 June 1993 (17.06.93)</td> <td>US</td> </tr> </table> <p>(71) Applicant: THE SCRIPPS RESEARCH INSTITUTE [US/US]; 10666 North Torrey Pines Road, La Jolla, CA 92037 (US).</p> <p>(72) Inventors: YUAN, Wei ; 8155 Cargill Avenue, San Diego, CA 92122 (US). WONG, Chi-Huey ; 6399 Calle Del Alcazar, Rancho Sante Fe, CA 92067 (US). SAMUELS-SON, Bengt ; Framnasvagen 6, S-182 63 Djursholm (SE). MUNOZ, Benito ; 6190 Shoreline Drive, San Diego, CA 92122 (US).</p>	07/900,959	18 June 1992 (18.06.92)	US	07/995,789	23 December 1992 (23.12.92)	US	08/079,239	17 June 1993 (17.06.93)	US	<p>(74) Agents: GAMSON, Edward, P.; Dressler, Goldsmith, Shore, Sutker & Milnamow, Ltd., 4700 Two Prudential Plaza, 180 North Stetson Avenue, (US) et al.</p> <p>(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
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<p>(54) Title: NOVEL TIGHT-BINDING INHIBITORS OF LEUKOTRIENE A₄ HYDROLASE</p> <div style="text-align: center; margin: 20px 0;">  <p>(I)</p> </div> <p>(57) Abstract</p> <p>Inhibitors of leukotriene A₄ hydrolase are disclosed, as are processes for making and using the same. A contemplated inhibitor has a structure corresponding to formula (I), wherein the depicted -NH₂ group is in the (S) configuration; -W is -CH₂SH, -CH₂NH₂ or C(=Z)-Y, wherein =Z is =O, or -H and -OH; and -Y is selected from the group consisting of (a) phenyl, (b) trifluoromethylphenyl, (c) carboxyphenyl, (d) benzyl, (e) C₁-C₆ alkylencarboxyl, (f) C₁-C₆ alkyl, (g) C₂-C₆ alkenyl, (h) C₁-C₆ alkylenephenyl and (i) -C(=O)-X-R¹ wherein X is O or NH and, R¹ is selected from the group consisting of C₁-C₆ alkyl, C₁-C₆ alkylencarboxyl, and benzyl, with the proviso that -Y is other than (i) when =Z is -H and -OH; and R² is hydrogen, benzyloxy or 2-naphthylmethyloxy, and a pharmaceutically acceptable acid addition salt thereof.</p>										

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NOVEL TIGHT-BINDING INHIBITORS OF
LEUKOTRIENE A₄ HYDROLASE

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Description

Technical Field

The present invention relates to inhibitors of the enzyme leukotriene A₄ (LTA₄) hydrolase, and particularly to α -keto- or α -hydroxy- β -amino-phenylbutyric acid ester and amide compounds, as well as β -amino-phenylpropyl mercaptans and amines.

Background Art

Leukotriene (LT) A₄ hydrolase (EC 3.3.2.6) [Samuelsson et al., J. Biol. Chem., 264:19469 (1989)] is a zinc-containing monomeric enzyme (MW ~ 70 kD) [Haeggstrom et al., Biochem. Biophys. Res. Commun., 172:965 (1990)] that exhibits both epoxide hydrolase and aminopeptidase [Haeggstrom et al., Biochem. Biophys. Res. Commun., 173:431 (1990); Minami et al., Biochem. Biophys. Res. Commun., 173:620 (1990)] activities. The enzyme catalyzes the formation of the inflammatory mediator leukotriene (LT) B₄ (5S,12R-dihydroxy-6,14-cis-8,10-trans-eicosatetraenoic acid) from its natural substrate leukotriene (LT) A₄ (5(S)-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid), one of the physiologically important processes in the arachidonic acid biosynthetic pathway. [Samuelsson et al., J. Biol. Chem., 264:19469 (1989).]

The enzyme has been purified to homogeneity from various sources as a water soluble protein [Samuelsson et al., J. Biol. Chem., 264:19469 (1989)] and the genes coding for the human enzyme from placenta and spleen have been cloned and sequenced. Funk et al., Proc.

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Natl. Acad. Sci. USA, 84:6671 (1987). Although the detailed mechanism of the enzyme has not been elucidated, it is thought that the addition of a water molecule to the enzyme's substrate is general base-assisted and the role of the zinc ion is to serve as a Lewis acid to polarize epoxide ring or the carbonyl of amide, and to stabilize the negative charge developed during the reaction.

The zinc ion may also bind to the nucleophilic water molecule to facilitate the general-base catalysis. The peptidase and epoxide hydrolase activities, which occur at the same active site, may use a different general base (a carboxylate residue) as indicated in recent site-directed mutagenesis studies [Samuelsson et al., Proc. Natl. Acad. Sci. USA, 89:9141 (1992)]. Both enzymatic activities are activated by albumin, a characteristic distinguishing this enzyme from other Zn^{++} metaldohydrolases. [Oring et al., Biochemistry, 31:4218-4223 (1992).]

It is of great interest to develop selective inhibitors of LTA_4 hydrolase as potential antiinflammatory agents since LTB_4 is a strong proinflammatory mediator that stimulates adhesion of circulating neutrophils to vascular endothelium [McIntyre et al., Proc. Natl. Acad. Sci. USA, 83:2204 (1986)] and directs neutrophil migration toward sites of inflammation. [Ford-Hutchinson et al., Nature, 286, 264 (1980)]. LTA_4 hydrolase was irreversibly inhibited by its substrate LTA_4 [McGee et al., J. Biol. Chem., 260:12832 (1985)] and substrate analogues LTA_3 and LTA_5 , [Evans et al., J. Biol. Chem., 269:10966 (1985); Nathaniel et al, Biochem. Biophys. Res. Commun., 131:827 (1985); Ohoshi et al., J. Biol. Chem., 262:10200 (1987)]. In addition, some inhibitors of Zn^{++} -containing amino peptidase (e.g. Bestatin) and

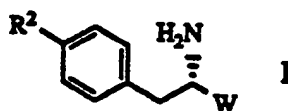
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angiotensin converting enzyme (e.g. Captopril) are reversible inhibitors of LTA₄ hydrolase. Orning et al., J. Biol. Chem., 266:1375 (1991).

Our study on the specificity of the amidase activities of LTA₄ hydrolase and the synthesis of a series of α -keto- or α -hydroxy- β -amino acid esters and amides and peptide isosteres for use to inhibit the activity of the enzyme are discussed hereinafter. This study has led to the development of a new class of transition state analog inhibitors based on both the proposed mechanism of the aminopeptidase activity and the natural substrate structure of the enzyme.

Brief Summary of the Invention

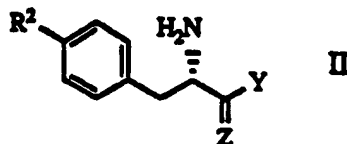
An inhibitor compound of the invention corresponds in structure to Formula I, below:



wherein $-W$ is $-CH_2SH$, $-CH_2NH_2$ or $-C(=Z)-Y$, wherein $=Z$ is $=O$, or $-H$ and $-OH$; and $-Y$ is selected from the group consisting of (a) phenyl, (b) trifluoromethylphenyl, (c) carboxyphenyl, (d) benzyl, (e) C_1-C_6 alkylencarboxyl, (f) C_1-C_6 alkyl, (g) C_2-C_6 alkenyl, (h) C_1-C_6 alkylenephenyl and (i) $-C(=O)-X-R^1$ wherein X is O or NH , and R^1 is selected from the group consisting of C_1-C_6 alkyl, C_1-C_6 alkylencarboxyl and benzyl; and R^2 is hydrogen, benzyloxy or 2-naphthylmethyloxy. The amine group ($-NH_2$) depicted in Formula I has the S configuration.

Particularly preferred compounds of Formula I are those wherein W is $-CH_2SH$ or $-CH_2NH_2$, and those of Formula II, below, wherein $-W$ is $-C(=Z)-Y$, as discussed above.

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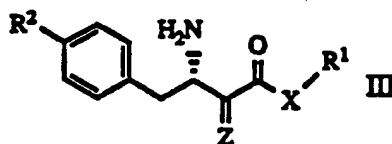


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wherein R^2 , $=Z$ and $-Y$ are as discussed before.

Another preferred compound of Formula II are those wherein $-Y$ is (a)-(h) above, and where $-Y$ is (i); i.e., $-C(=O)-X-R^1$. Where $-Y$ is $-C(=O)-X-R^1$, a compound of Formula III, below, results

10



15

wherein the depicted $-NH_2$ group has the (S) configuration;

X is O or NH;

R^1 is selected from the group consisting of C_1-C_6 alkyl, C_1-C_6 alkylencarboxyl and benzyl;

20

R^2 is hydrogen (H), benzyloxy or 2-naphthylmethyloxy; and

$=Z$ is $=O$ or $-H$ and $-OH$.

A pharmaceutically acceptable acid addition salt of any of the above compounds of Formulas I, II or III is also contemplated.

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In preferred practice, X is O. R^1 is preferably C_1-C_6 alkyl, or benzyl and R^2 is preferably benzyloxy.

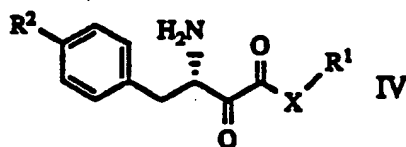
An inhibitor compound more preferably contains at least two phenyl rings, and most preferably contains three phenyl rings. Thus, when R^1 is other than benzyl, R^2 is more preferably benzyloxy or 2-naphthylmethyloxy. Most preferably, an inhibitor compound contains an R^1 benzyl group and an R^2 benzyloxy group. Where $=Z$ contains single bonds to a hydrogen and a hydroxyl, it

35

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is preferred that the hydroxyl group have the (S) configuration, so that preferred α -hydroxy- β -amino inhibitor compounds have the (2S,3S) configuration.

5 More preferably, $=Z$ is $=O$ so the compound is an α -keto- β -amino acid ester or amide, with an ester being preferred over an amide. A more preferred compound corresponds in structure to Formula IV, below, wherein R^1 , R^2 and X are as above, with the above preferences. A pharmaceutically acceptable acid addition salt of a
10 compound of Formula IV is also contemplated.



15 A pharmaceutical composition containing leukotriene A_4 hydrolase-inhibiting amount of a before-described compound dissolved or dispersed in a pharmaceutically acceptable diluent is also
20 contemplated.

A process for inhibiting the activity of a leukotriene A_4 hydrolase is also contemplated. Here, the process is carried out by admixing the enzyme, its substrate and an inhibiting amount of a before-described
25 inhibitor compound in an aqueous medium under biochemical reaction conditions, and maintaining that admixture under those conditions for a time period sufficient for the enzyme's activity to be inhibited. The inhibitor compound is preferably supplied via an
30 above composition.

Detailed Description

I. The Compounds

35 An inhibitor compound of the present invention can be viewed as a derivative of 2-amino-3-

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phenylpropane. Each compound has the 2-amino (or α -amino) group in the (S) configuration.

One embodiment of a contemplated inhibitor is (2S)-2-amino-3-phenylpropyl mercaptan or its
5 4'-benzylether. The latter, 2-amino-3-(4'-benzyloxy)phenylpropyl mercaptan, Compound 27, is a most preferred compound herein, as is its dimer Compound 27a. Another contemplated inhibitor is (2S)-3-[4-(2-naphthylmethyloxy)phenyl]-1,2-diamino-propane HCl,
10 Compound 60, as is the corresponding 4'-benzyloxy compound, Compound 61.

In other embodiments of the invention, the 1-position of the propyl group is derivatized as a carbonyl group (C=O) or as a hydroxyl group (OH), with
15 still further groups bonded to the remaining valence of the 1-position carbon atom. In a compound of these embodiments, the propyl 1-position carbon atom is shown as C(=Z)-Y, wherein =Z is the carbonyl oxygen (=O) or hydrogen and hydroxyl (-H and -OH), and -Y is the group
20 that is bonded to the remaining valence of the propyl 1-position carbon atom.

In one aspect, -Y is a carboxylic acid ester or amide group, making the contemplated inhibitor or derivative of 3-amino-2-hydroxy-4-phenylbutanoic acid
25 (APHA) where =Z is -H and -OH, or 3-amino-2-oxo-4-phenylbutanoic acid where =Z is =O. The 2-hydroxyl group of an APHA derivative can be in either (2S) or (2R) configuration, with the (2S) configuration being preferred. It is more preferred that the 2-hydroxyl
30 group of such a derivative be oxidized to a keto or oxo group. When viewed as a 4-phenylbutanoic acid derivative, the before-mentioned (S)-configured amine group is in the 3-position and can be referred to as a β -amino group.

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Thus, a contemplated later-discussed inhibitor compound is a (3S)-amino-2(R or S)-hydroxy-4-phenylbutanoic acid ester or amide or a (3S)-amino-2-keto-4-phenylbutanoic acid ester or amide. The 2- and 3-positions are also referred to herein as the α - and β -positions of the molecule, respectively.

The 4-phenyl portion of APHA can be a phenyl group itself, can be a 4'-(benzyloxy)-phenyl group or a 4'-(2-naphthylmethyloxy) group. Thus, the 4-phenyl group can have a 4'-substituent, referred to hereinafter as R^2 in the structural formulas that follow that can be hydrogen (H), benzyloxy or 2-naphthylmethyloxy. A 2-naphthylmethyloxy group has the structure $-O-CH_2-$ naphthalene, where the $-CH_2-$ group is bonded to the 2-position of the naphthyl ring.

Because carboxylic acid ester or amide such as a contemplated APHA derivative can be the reaction product of a carboxylic acid and an alcohol or amine, the carbon-containing group derived from the alcohol or amine can be referred to as the alcohol or amine portion of the ester or amide, respectively. That alcohol or amine portion, referred to as R^1 in Formulas I-IV and VII-VIII hereinafter, can be selected from the group consisting of C_1-C_6 alkyl (as above), carboxyl C_1-C_6 alkyl, and benzyl.

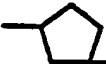
C_1-C_6 Alkyl groups are well known and include methyl, ethyl, isopropyl, sec-butyl, cyclopentyl, hexyl, 2-methylpentyl, cyclohexyl and the like.

A carboxyl C_1-C_6 alkyl group is an alkyl group as before-discussed that itself further includes a substituent carboxyl group. A carboxyl C_1-C_6 alkyl group can also more precisely be referred to as a C_1-C_6 alkylencarboxyl group to clarify that the alkylene group is bonded to the oxygen or nitrogen atom of the ester or amide and to the carboxyl group. The

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contemplated groups include the α -carbon, carboxyl and side groups of the naturally occurring amino acids as well as their D-isomers. Indeed, a D- or L-amino acid can be used to form a contemplated amide inhibitor.

5 Contemplated C_1 - C_6 alkylencarboxyl groups thus include carboxymethyl (carboxymethylene; $-\text{CH}_2\text{CO}_2\text{H}$), carboxyethyl (carboxyethylene; $-\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 2-carboxybutyl [2-carboxybutylene; $-\text{CH}_2-\text{CH}(\text{CO}_2\text{H})\text{C}_2\text{H}_5$], 3-carboxycyclopentyl (3-carboxycyclopentylene;

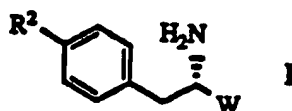
10  CO_2H) and the like.

In the embodiments noted before wherein the
15 1-position of the propyl group is derivatized as a carbonyl group or as a hydrogen and hydroxyl group with still further groups bonded to the 1-position; i.e., where that propyl 1-position carbon is $\text{C}(=\text{Z})-\text{Y}$, those further groups can be selected from a number of
20 moieties. Exemplary of those moieties are phenyl, trifluoromethyl, carboxyphenyl, benzyl, C_1 - C_6 alkylencarboxyl, C_1 - C_6 alkyl, C_2 - C_6 alkenyl and C_1 - C_6 alkylenophenyl.

The trifluoromethyl and carboxyphenyl groups can
25 be substituted in the ortho, meta or para positions of the phenyl ring, but meta and para are preferred. C_1 - C_6 Alkyl and C_1 - C_6 alkylencarboxy groups are as discussed before. A C_2 - C_6 alkenyl group is an ethyleneically unsaturated C_2 - C_6 alkyl group. Exemplary groups include
30 ethylenyl, 2-propylenyl, 4-pentylenyl, cyclohex-3-enyl and the like. A C_1 - C_6 alkylenophenyl group is C_1 - C_6 alkylene group bonded to the carbon shown in $\text{C}(=\text{Z})-\text{Y}$, the 1-position carbon atom, and to a phenyl ring.

Thus, a preferred contemplated inhibitor
35 compound has a structure corresponding to Formula I, below,

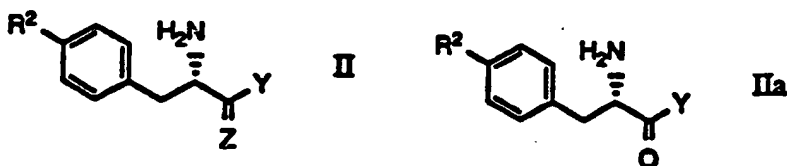
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wherein -W is -CH₂SH, -CH₂NH₂ or -C(=Z)-Y, wherein =Z is =O, or -H and -OH; and -Y is selected from the group consisting of (a) phenyl, (b) trifluoromethylphenyl, (c) carboxyphenyl, (d) benzyl, (e) C₁-C₆ alkylencarboxyl, (f) C₁-C₆ alkyl, (g) C₂-C₆ alkenyl, (h) C₁-C₆ alkylenephenyl and (i) -C(=O)-X-R¹ wherein X is O or NH, and R¹ is selected from the group consisting of C₁-C₆ alkyl, C₁-C₆ alkylencarboxyl and benzyl; and R² is hydrogen, benzyloxy or 2-naphthylmethyloxy. The amine group (-NH₂) depicted in Formula I has the S configuration.

Particularly preferred compounds of Formula I are those wherein -W is -CH₂SH or -CH₂NH₂, and those of Formula II, below, wherein -W is -C(=Z)-Y, as discussed above,



25

wherein R², =Z and -Y are as discussed before, with R² preferably being other than hydrogen and =Z preferably being =O. -Y is most preferably C₁-C₆ alkylenephenyl. Compounds that include the structural preferences above for Formula II are included among those of Formula IIa, above.

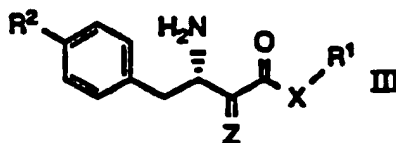
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Another group of preferred compounds of Formula II are those wherein -Y is (a)-(g) above, and where -Y

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is (h); i.e., $-C(=O)-X-R^1$. Where $-Y$ is $-C(=O)-X-R^1$, a compound of Formula III, below, results

5



wherein $=Z$ is $=O$, or $-H$ and $-OH$;

10

X is O or NH , and R^1 is selected from the group consisting of C_1-C_6 alkyl, C_1-C_6 alkylencarboxyl and benzyl; and

R^2 is hydrogen, benzyloxy or 2-naphthylmethyloxy.

15

Where $=Z$ is $-H$ and $-OH$, it is preferred that the $-OH$ group have the (S) configuration. Of the R^1 groups, benzyl is presently preferred, whereas benzyloxy is presently preferred as the R^2 group.

20

In examining the above structural formulas it is noted that $=Z$ can be one of two entities; i.e., a doubly bonded oxygen (an oxo group) or a hydroxyl and a hydrogen. Thus, the two bonds shown to Z indicate the two valences from the carbon atom taken up by the Z entity, and not necessarily a double bond, except where $=Z$ is an oxo group.

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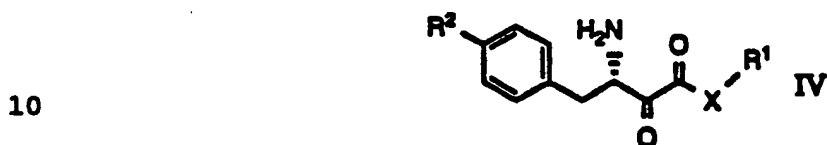
It is more preferred that an inhibitor compound contain at least two phenyl rings, four such rings being permitted by a prior formula in which the naphthalene-containing moiety is counted as two phenyl rings. It is most preferred that an inhibitor contain at least three phenyl rings. Thus, it is more preferred that when R^1 is other than benzyl, that R^2 be benzyloxy or 2-naphthylmethyloxy. Most preferably, an inhibitor compound contains both an R^1 benzyl group and an R^2 benzyloxy group so that the inhibitor contains three

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phenyl rings. Esters; i.e., compounds where X is O, are also presently preferred over amides.

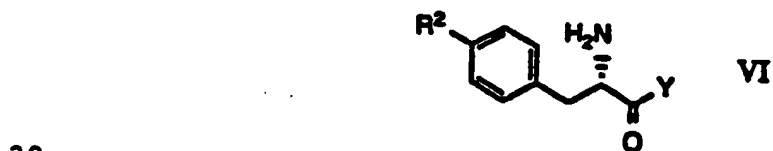
5 A more preferred compound is an α -keto- β -amino compound derivative of APHA whose structure corresponds to Formula IV, below, wherein R¹, R² and X are as above, with the same preferences for those groups noted above.



15 Following the preference for R² being benzyloxy, a preferred inhibitor of the invention has a structure that corresponds to Formula V, below, wherein W is as defined before.

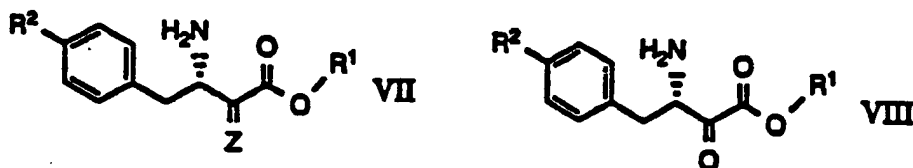


25 Following the preference that =Z be =O, a particularly preferred compound having a structure corresponding to Formula II has a structure corresponding to Formula VI, below, wherein -Y is as defined before.



35 Following the preference that X be oxygen, O, another group of particularly preferred inhibitors of Formulas III and IV has a structure of Formulas VII and VIII, respectively, below, wherein R¹, R² and =Z are as before defined.

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5 An inhibitor compound can also be present as a
 pharmaceutically acceptable acid addition salt.
 Typically, where the ester or amide is electrically
 neutral at physiological pH values, e.g. pH 7.2-7.6, or
 at another pH value at which it is utilized or prepared,
 10 the β -amino group is present as an acid addition salt.
 Exemplary acids from which the salts can be prepared are
 well known to workers skilled in the art and include
 HCl, HBr, HI, H_2SO_4 , NaHSO_4 , H_3PO_4 , KH_2PO_4 , $\text{C}_1\text{-C}_{18}$
 carboxylic acids such as formic, acetic, butyric,
 15 maleic, fumaric, oleic, stearic, palmitic, lactic,
 citric, and tartaric acids as well as benzoic acid.
 Where R^1 includes a carboxyl group, a compound is
 typically present as a zwitterion.

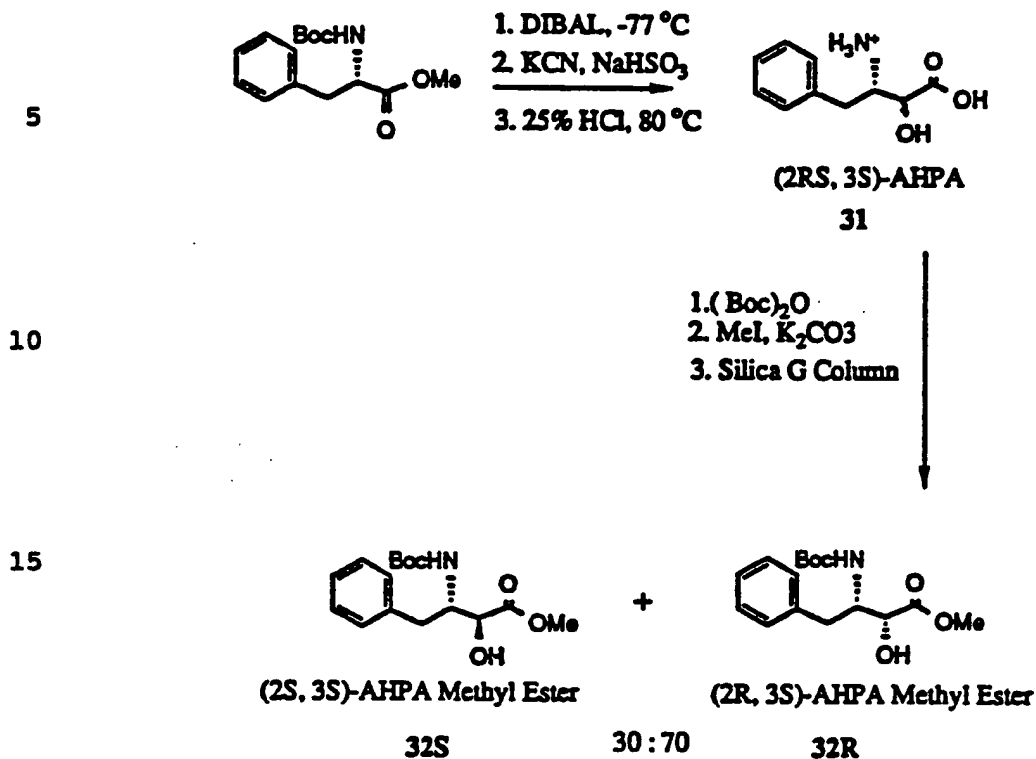
20 II. Compound Syntheses

Syntheses of an inhibitor described herein is
 straightforward. Detailed syntheses for particular
 compounds, or physical data for those compounds are
 provided hereinafter.

25 α -Hydroxy- β -amino acids were prepared from the
 corresponding α -amino acids. A representative synthesis
 of N-t-butyloxycarbonyl-(2RS,3S)-3-amino-2-hydroxy-
 phenylbutanoic acid [abbreviated as N-BOC-(2RS,3S)-APHA]
 methyl esters Compound 32S and 32R is illustrated in
 30 Scheme 1, below.

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Scheme 1



25

Thus, (2RS,3S)-APHA (Compound 31) was converted to N-BOC-(2RS,3S)-APHA methyl ester [Yuan et al., Bioorg. & Med. Chem. Lett., 1:551 (1991)] and the diastereomers were separated on silica gel to give N-BOC-(2R,3S)-AHPA and N-BOC-(2S,3S)-AHPA methyl esters Compounds 32S and 32R. The stereochemistry at the 2-position was assigned by comparison of (2S,3R)-AHPA isopropyl ester to the published data. Iizuka et al., J. Med. Chem., 33:2707 (1990).

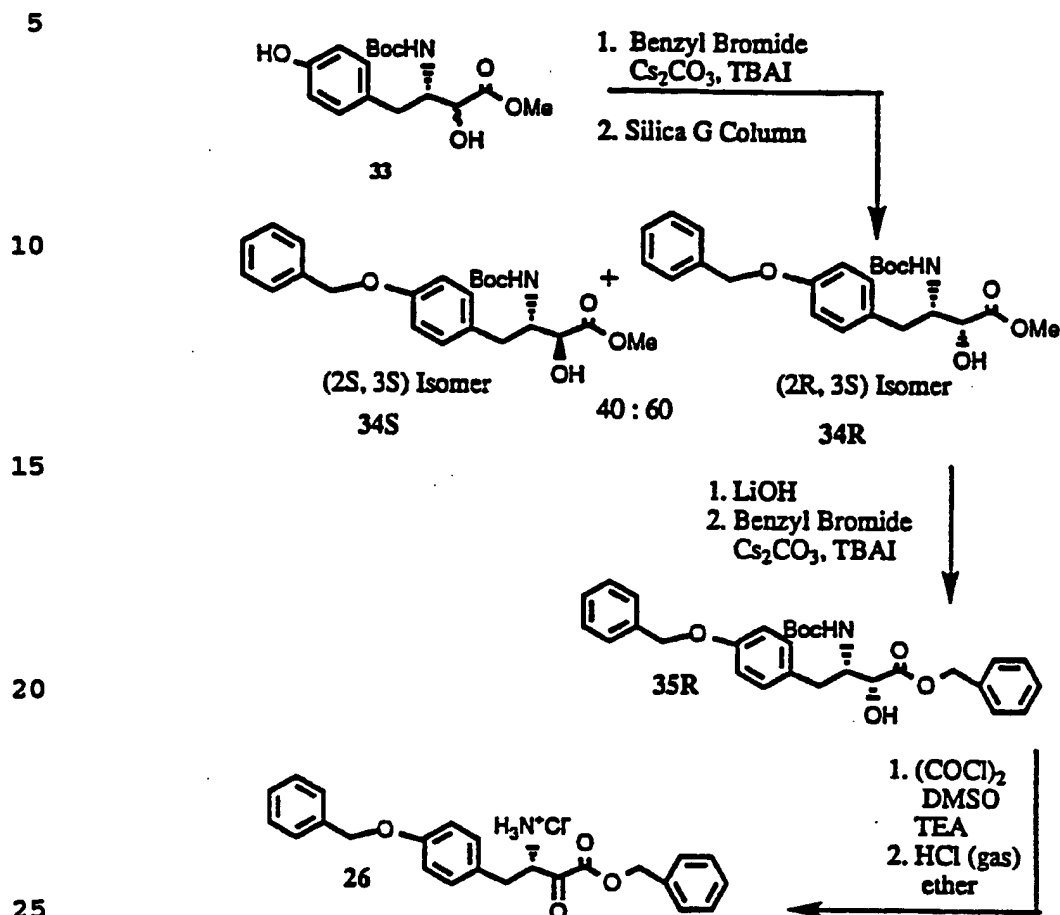
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Similar procedures were followed to prepare (Compound 34S) (the configuration of the 2-position was assigned tentatively with confidence based on the inhibitory activities of two isomers) and Compound 34R in Scheme 2, below. Compound 34R was then converted

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into Compound 35R, and thereafter into Compound 26, as is also shown in Scheme 2.

Scheme 2



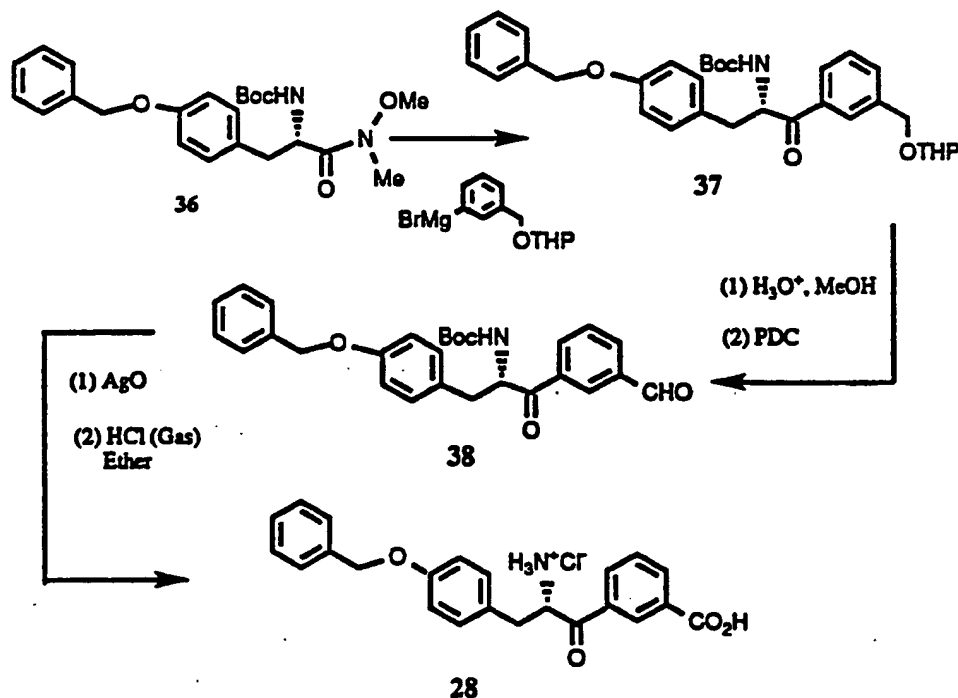
Other esters and amides contemplated herein can be prepared using Compound 34R as a starting material using standard ester-alcohol or ester-amine interchange reactions in which the alcohol portion of an ester is replaced by another alcohol or amine, respectively, to form a corresponding new ester or amide. The resulting compounds that are analogous in structure to Compound 35R can then be oxidized and deblocked to form a preferred α -keto- β -amine inhibitor compound.

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The dipeptides (Compounds 7, 8, 9 and 10) were made by DCC-mediated coupling of the corresponding carboxylic acids followed by deprotection. The α -keto Compounds 21, 22, 25 and 26 were prepared from the corresponding alcohol via Swern oxidation [Mancuso et al., *J. Org. Chem.*, 43:2480 (1978)] followed by deprotection, as noted before in Scheme 2 for Compound 26.

Contemplated inhibitor compounds where -Y is other than a carboxylic acid, ester or amide can be prepared using the chemistry outlined below in Schemes 3 or 4, below beginning with Weinreb amide Compounds 36 or 39. [Nahm et al., *Tetrahedron Lett.*, 22:3815-3818 (1981); Angelastro et al., *J. Org. Chem.*, 54:3913-3916 (1989).] With these syntheses, the Weinreb amide is first recited with a Grignard reagent to form the keto group that is adjacent to the S-configured amino group.

Scheme 3

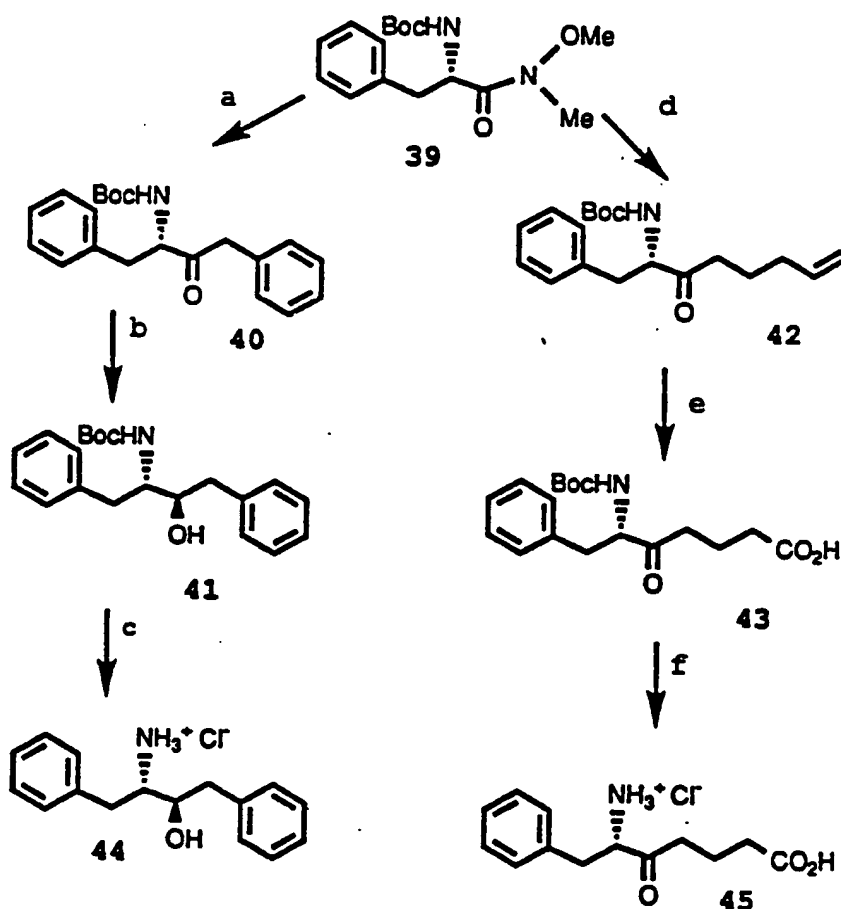


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In Scheme 3 the Grignard reagent included a marked or blocked functional group, a tetrahydropyranyl (THP)-blocked benzyl alcohol. The THP group of adduct Compound 37 was removed by use of an acid (HCl) in methanol, and the resulting Boc-protected benzyl alcohol was oxidized to the aldehyde of Compound 38 using pyridinium dichromate (PDC). The Boc-protected aldehyde of Compound 38 was then oxidized with silver oxide to form the corresponding phenyl carboxylic acid, and that carboxylic acid treated with HCl gas in ether to remove the Boc group and form the HCl salt of Compound 28.

Scheme 4 is shown below.

Scheme 4



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As is seen from Scheme 4, the Weinreb amide Compound 39 (N-Boc-L-phenylalanine-N-methoxy-N-methylamide) was the starting material for two specific inhibitors herein, Compounds 44 and 45. Thus, enroute to Compound 44, Compound 39 was reacted with benzyl magnesium chloride in step a to form Compound 40 and 98 percent yield. Compound 40 was reduced in step b with NaBH₄ in anhydrous methanol (MeOH) at -23°C to provide Compound 41 in 80 percent yield. Compound 41 was deblocked with trifluoroacetic acid (TFA) and then treated with 1N HCl from which the HCl salt of Compound 44 was obtained in 88 percent yield in step c.

On the right-hand side of Scheme 4, Compound 39 was reacted with pentene magnesium bromide in step d to form Compound 43 in 97 percent yield. A deblocked derivative of Compound 42 is a compound of the invention wherein -Y is C₂-C₆ alkenyl. Oxidation of Compound 43 in step e with NaIO₄ and KMnO₄ provided Compound 43 in 78 percent yield. Compound 43 was converted to Compound 45 as discussed for the conversion of Compound 41 to Compound 44, in 88 percent yield in step f.

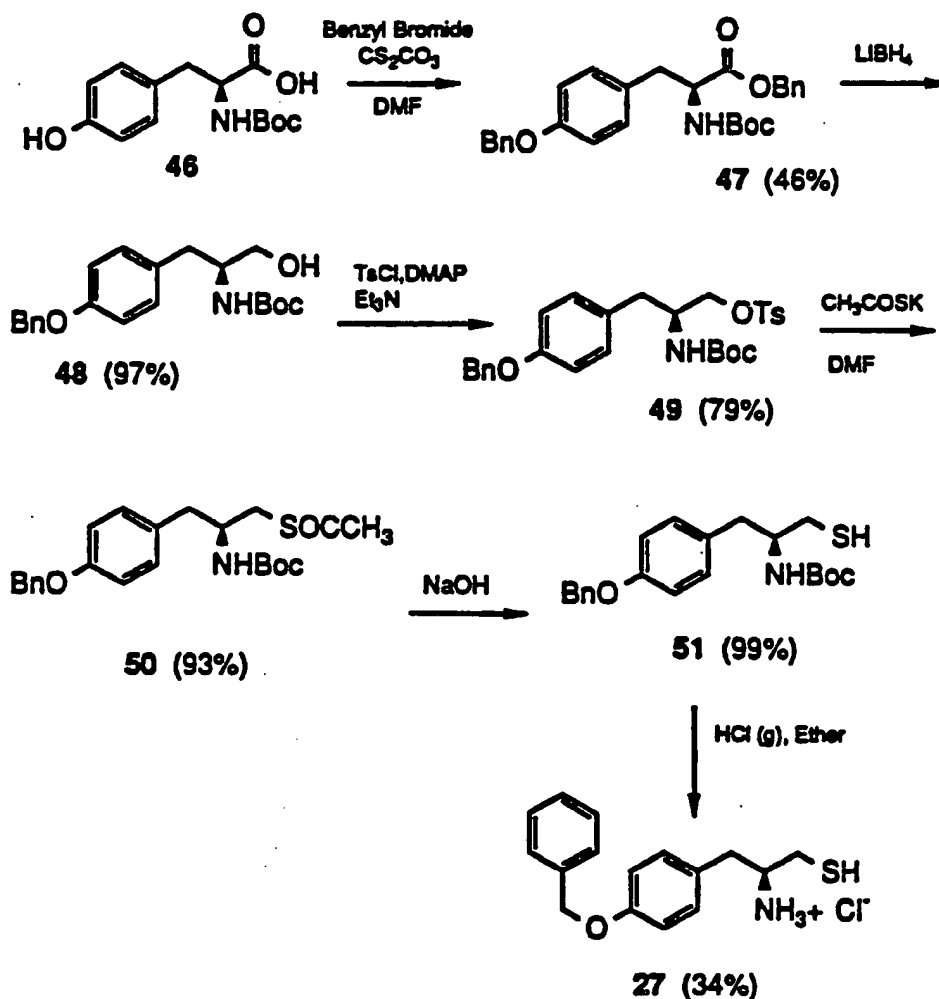
A similar reaction to those shown in Scheme 4 was used to prepare Compound 54, shown hereinafter. This reaction started with the Weinreb amide of 4'-(benzyloxy)-N-Boc-L-tyrosine (Compound 52) that was reacted in THF with 3-phenylpropane magnesium bromide to form the corresponding N-Boc phenyl-substituted ketone, Compound 53. Removal of the Boc group with HCl gas in ether provided Compound 54.

An exemplary synthesis for Compound 26 is shown in Scheme 5, hereinafter. Thus, N-Boc-L-tyrosine (Compound 46) was reacted with benzyl bromide in DMF provide Compound 47, which was recovered, dissolved in THF and reduced with LiBH₄ to provide Compound 48. Reaction of Compound 48 in dichloromethane with tosyl

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chloride (TsCl) and a catalytic amount of DMAP and triethylamine provided tosylate Compound 49. That compound was reacted with potassium thioacetate in DMF to provide thioester Compound 50, whose acetyl group was cleaved with hydroxide in ethanol to provide the N-Boc mercaptan Compound 52. Removal of the N-Boc blocking group with HCl gas in ether provided Compound 27.

Scheme 5

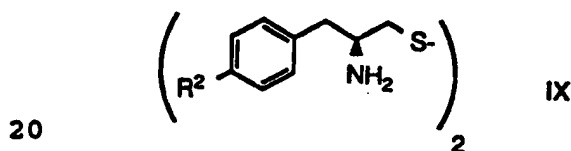


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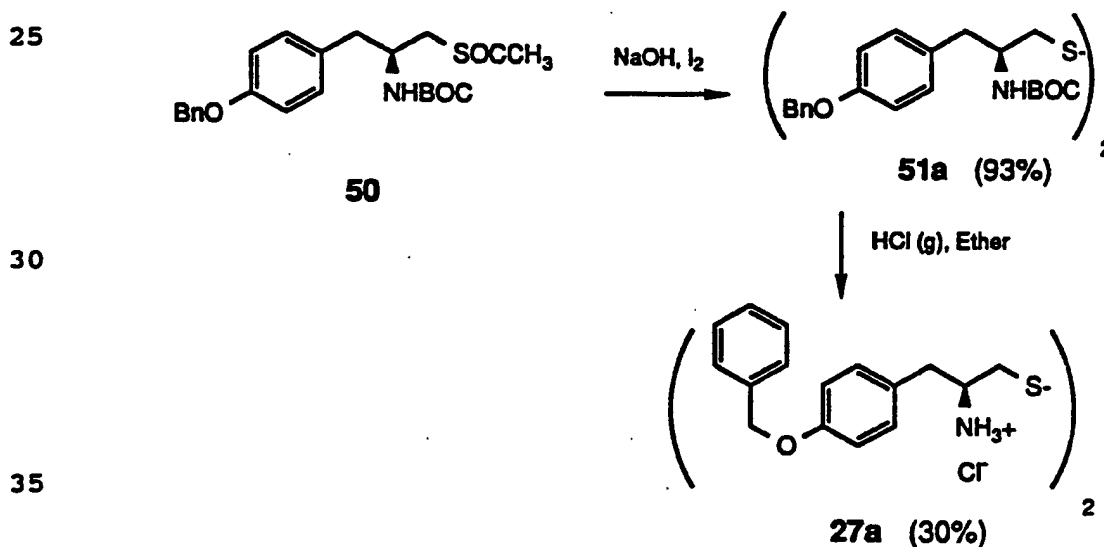
The disulfide of Compound 27, Compound 27a, is also useful herein as a prodrug form of Compound 27. Thus, administration of Compound 27a in vitro in the presence of a mild reductant such as dithiothreitol provides Compound 27. Similarly, administration of Compound 27a in vivo provides Compound 27 by body fluid reductants.

Compound 27a was prepared from Compound 50, by cleavage of the acetyl group and oxidation of the mercaptan with iodine (I_2) in ethanol (EtOH) to provide the disulfide Compound 51a. Compound 51a was then deblocked to provide Compound 27a. These steps are shown in Scheme 5a, below.

More generally, Compound 27a is one of group of compounds whose structures correspond to Formula IX, below, where R^2 is as before described.



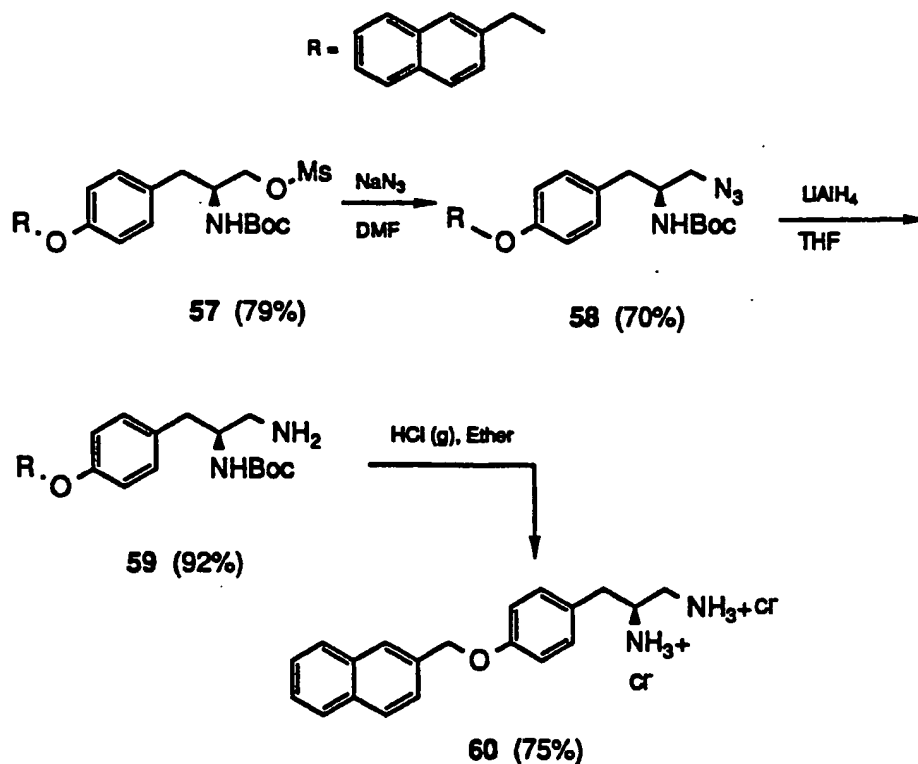
Scheme 5a



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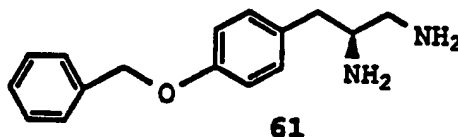
Scheme 6, hereinafter, illustrates the synthesis of Compound 60. Compound 57 was prepared in a manner similar to that discussed before for Compound 49, except that 2-(bromomethyl)naphthalene was used instead of benzyl bromide to provide a 2-naphthylmethoxy group (R), and mesyl chloride was used instead of tosyl chloride to provide a mesylate group (Ms). Compound 57 was reacted with sodium azide in DMF to provide Compound 58, whose azido group was reduced with LiAlH_4 in THF to provide the N-Boc amino compound, Compound 59. The Boc group was removed as before to provide the diamino hydrochloride (Compound 60).

Scheme 6



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Compound 61, below, can readily be prepared from Compound 49 or its mesylate rather than tosylate derivative by reaction with sodium azide in DMF, followed by LiAlH_4 reduction and treatment of the resulting product with HCl gas in ether.



It should be readily apparent that the remaining compounds of Formulas I-VIII can be prepared by use of the reactions of the above schemes and analogous reactions to those shown, all of which are well known to skilled workers in organic synthesis.

III. Pharmaceutical Compositions

A pharmaceutical composition is contemplated that contains a before-described compound or its acid addition salt of the invention as active agent. A pharmaceutical composition is prepared by any of the methods well known in the art of pharmacy all of which involve bringing into association the active compound and the carrier therefor. For therapeutic use, a compound or salt of the present invention can be administered in the form of conventional pharmaceutical compositions. Such compositions can be formulated so as to be suitable for oral or parenteral administration, or as suppositories. In these compositions, the agent is typically dissolved or dispersed in a physiologically tolerable carrier or diluent.

A carrier or diluent is a material useful for administering the active compound and is "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. As used

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herein, the phrases "physiologically tolerable" and "pharmaceutically acceptable" are used interchangeably and refer to molecular entities and compositions that do not produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a mammal. The physiologically tolerable carrier and diluent can take a wide variety of forms depending upon the preparation desired for administration and the intended route of administration.

As an example of a useful composition, a compound or salt of the invention (active agent) can be utilized, dissolved or dispersed in a liquid composition such as a sterile suspension or solution, or as an isotonic preparation containing suitable preservatives. Particularly well-suited for the present purposes are injectable media constituted by aqueous injectable buffered or unbuffered isotonic and sterile saline or glucose solutions, as well as water alone, or an aqueous ethanol solution. Additional liquid forms in which these compounds or salts can be incorporated for administration include flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, peanut oil, and the like, as well as elixirs and similar pharmaceutical vehicles. Exemplary further liquid diluents can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA (1980).

An active agent can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain stabilizers, preservatives,

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excipients, and the like in addition to the agent. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic.

5 Methods of forming liposomes are known in the art. See, for example, Prescott, Methods in cell Biology, Vol. XIV, Academic press, New York, N.Y. (1976), p.33 et seq.

10 An active agent can also be used in compositions such as tablets or pills, preferably containing a unit dose of the compound or salt. To this end, the agent (active ingredient) is mixed with conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate,
15 dicalcium phosphate, gums, or similar materials as non-toxic, physiologically tolerable carriers. The tablets or pills can be laminated or otherwise compounded to provide unit dosage forms affording prolonged or delayed action.

20 It should be understood that in addition to the aforementioned carrier ingredients the pharmaceutical formulation described herein can include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders,
25 surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient.

30 The tablets or pills are preferably also provided with an enteric layer in the form of an envelope that serves to resist disintegration in the stomach and permits the active ingredient to pass intact into the duodenum or to be delayed in release. A
35 variety of materials can be used for such enteric layers

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or coatings, including polymeric acids or mixtures of such acids with such materials as shellac, shellac and cetyl alcohol, cellulose acetate phthalate, and the like. A particularly suitable enteric coating comprises
5 a styrene-maleic acid copolymer together with known materials that contribute to the enteric properties of the coating. Methods for producing enteric coated tablets are described in U.S. Patent 4,079,125 to Sipos, which is herein incorporated by reference.

10 The term "unit dose", as used herein, refers to physically discrete units suitable as unitary dosages for administration to warm blooded animals, each such unit containing a predetermined quantity of the agent calculated to produce the desired therapeutic effect in
15 association with the pharmaceutically acceptable diluent. Examples of suitable unit dosage forms in accord with this invention are tablets, capsules, pills, powder packets, granules, wafers, cachets, teaspoonfuls, dropperfuls, ampules, vials, segregated multiples of any
20 of the foregoing, and the like.

Actual dosage levels of an inhibitor compound or its salt in the compositions of the invention can be varied to obtain an amount of active ingredient that is effective to obtain a desired therapeutic response for a
25 particular composition and method of administration. The selected dosage level therefore depends upon the desired therapeutic effect, on the route of administration, on the desired duration of treatment and other factors.

30 Total daily dose of a contemplated compound or its salt administered to a host in a process discussed hereinafter in single or divided doses can be in amounts, for example, of from about 0.001 to about 100 mg/kg body weight daily and preferably 0.01 to 10
35 mg/kg/day. Unit dosage compositions can contain amounts

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of submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient or host mammal such as a mouse, rat or rabbit will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other drugs and the severity of the condition being treated.

For in vitro studies where the enzyme is present at about 1.4 $\mu\text{g/mL}$, final concentrations of about 1 mM to about 0.01 μM can be used, with higher concentrations being used in the pharmaceutical composition as desired to the limits of solubility or dispersibility. It should also be understood by a skilled worker that buffer and other salts that would not be pharmaceutically acceptable for use in vivo can be used in a pharmaceutical composition that is utilized for in vitro studies.

IV. Processes

A process of inhibiting the activity of leukotriene A_4 (LTA_4) hydrolase is also contemplated. In accordance with that process, that enzyme, its substrate and an inhibitor compound disclosed herein are admixed in an aqueous composition under biochemical reaction conditions, with the inhibitor compound being present in an amount sufficient to inhibit that enzyme. The resulting admixture is maintained under those conditions for a time period sufficient for the LTA_4 hydrolase to be inhibited.

Where enzyme inhibition is carried out in vitro, the aqueous composition is typically water containing appropriate buffers and other salts, if desired, that do not themselves inhibit the enzyme and are conducive to a

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reaction catalyzed by leukotriene A₄ hydrolase, as are well known. The temperature at which the inhibition is studied can be from above the freezing point of the resulting admixture to a temperature below the denaturation temperature of the enzyme. Typically, the temperature is about 10° to about 40°C, with ambient room temperature of about 20°-25°C most usually being used. The in vitro pH value usually used is between about 6.5 and about 8.5, with a pH value of about 7.5 to about 8.0 being preferred. The above conditions of buffer, salt concentration (osmolality), pH value and temperature are well known in biochemistry and are referred to herein as biochemical reaction conditions.

Typical in vitro maintenance times depend upon the enzyme and substrate concentrations and upon the specific biochemical reaction conditions utilized, as is well known for enzyme kinetics studies. Usual times for maintenance range from minutes to hours.

For in vitro use, inhibition of leukotriene A₄ hydrolase can be monitored by reaction with the enzyme's natural substrate, leukotriene A₄. More conveniently, however, inasmuch as the active site for the epoxide hydrolase and aminopeptidase activities are the same, a small molecule substrate, such as a p-nitroanilide of an amino acid can be used as the substrate and hydrolysis of the amide bond can be followed spectrophotometrically by production of yellow p-nitroaniline. An inhibition of at least about 25 percent of the enzyme's activity can be used as an endpoint in a study. More frequently, a 50 percent inhibition (IC₅₀) value is obtained. K_i value determinations provide another means for assaying inhibition.

For in vivo use, the temperature, pH value and osmolality (biological reaction conditions) are those of the host mammal to which the compound is administered.

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Inasmuch as the biochemistry of life for a given host mammal is carried out at those values, administration in vivo is also carried out under biochemical reaction conditions. Maintenance time in vivo is a function of the natural degradation and excretion times for a given host with a given inhibitor. Thus, the inhibitor is maintained within the host mammal such as a mouse, rat, rabbit, dog or human until it is consumed or excreted by natural bodily processes.

For in vivo use, inhibition of the activity of leukotriene A₄ hydrolase is monitored by the lessening of the degree of inflammation. Standard assays for the lessening of inflammation are well known and are exemplified in U.S. Patents No. 5,095,104; No. 4,687,781; No. 4,251,520; No. 3,784,701; No. 3,766,263; and No. 3,714,226, whose disclosures are incorporated by reference. Inasmuch as the product (LTB₄) of the enzyme's action on its substrate (LTA₄) stimulates migration of neutrophils toward sites of inflammation, well known assays for the relative numbers or amount of neutrophils at a site of inflammation can also provide evidence for inhibition of the enzyme's activity in vivo, as can assays for neutrophil chemotaxis. See, for example, U.S. Patent No. 5,095,104, whose disclosures are incorporated by reference.

In view of the effect of inhibiting the activity of LTA₄ hydrolase in vivo provides a lessened inflammatory response, another embodiment of the above process is a process for treating inflammation in a host mammal such as was noted before having inflammation. In accordance with this embodiment, a host mammal having inflammation is administered a LTA₄ hydrolase-inhibiting amount of a before-discussed inhibitor compound or its pharmaceutically acceptable salt to produce a reduction of the degree of inflammation in that host mammal. As

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noted previously, the amount administered in a single administration can be a submultiple of the total amount. In addition, multiple administrations over a period of several days, weeks or months are also contemplated, particularly for treating conditions of chronic inflammation such as arthritis.

Results

Several amino acid amides were assayed as substrates for LTA₄ hydrolase. Yuan et al., Bioorg. & Med. Chem. Lett., 1:551 (1991). It was found that only L-enantiomers were substrates, and L-alanine p-nitroanilide was the best (Table 1, below). D-Alanine p-nitroanilide was not acceptable.

Table 1

Comparison of Kinetic parameters to LTA₄ Hydrolase-Catalyzed Hydrolysis of LTA₄ and Amide Substrates

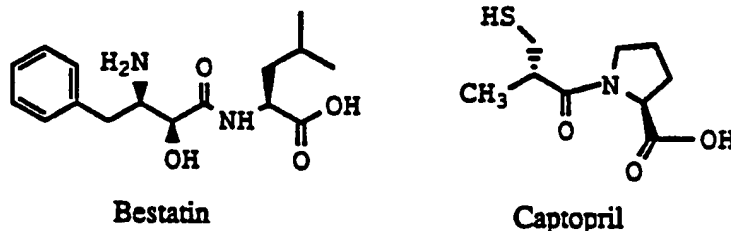
Substrate	K_m (μ M)	V_{max} (nmol/min/mg)	k_{cat}/K_m ($M^{-1}s^{-1}$)
LTA ₄	7.6 ^b	572 ^b	9.0×10^4
L-Lysine p-nitroanilide	100	30	3.5×10^2
L-Ala p-nitroanilide	500	530	1.2×10^3
D-Ala p-nitroanilide	---	0	---
L-Arg p-nitroanilide	200	135	7.5×10^2
L-Pro p-nitroanilide	100	135	1.5×10^3
L-Leu p-nitroanilide	300	135	5.0×10^2

^a Determined in 50 mM Tris-Cl, pH 7.6, in the presence of 0.1 M NaCl, $\epsilon_{410 \text{ nm}}$ for p-nitroaniline = $8850 \text{ M}^{-1}\text{cm}^{-1}$

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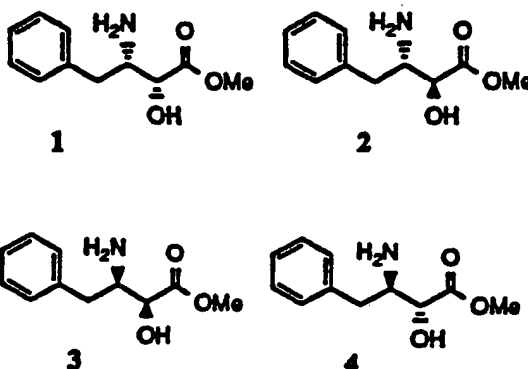
^b For native LTA₄ hydrolase, values of K_m and V_{max} ranging between 7-30 μM and 1.7-3.0 $\mu mol/min/mg$, respectively, have been reported using LTA₄ as substrate. Radmark et al., Adv. Prostaglandin, Thromboxane and Leukotriene Res., 20:35 (1990).

The inhibition activity of bestatin against LTA₄ hydrolase prompted the study of other L- and D-phenylalanine-derived norstatine-type of inhibitors. Without wishing to be bound by theory, it is thought that the aminopeptidase activity of LTA₄ hydrolase is mechanistically like that of Zn⁺⁺-containing thermolysin or aminopeptidase. The norstatine type of peptide isosteres, therefore, should be good inhibitors. The inhibition by Captopril may be due to a strong interaction between the SH group of the inhibitor and the zinc ion at the enzyme's active site.



Inhibitors having structures based on the proposed amidase activities were synthesized and evaluated against LTA₄ hydrolase purified from human leukocytes with L-alanyl p-nitroanilide as substrate. Initial studies [Yuan et al., Bioorg. & Med. Chem. Lett., 1:551 (1991)] of four 3-amino-2-hydroxy-4-phenylbutanoic acid (AHPA) methyl ester stereoisomers, Compounds 1, 2, 3 and 4, below,

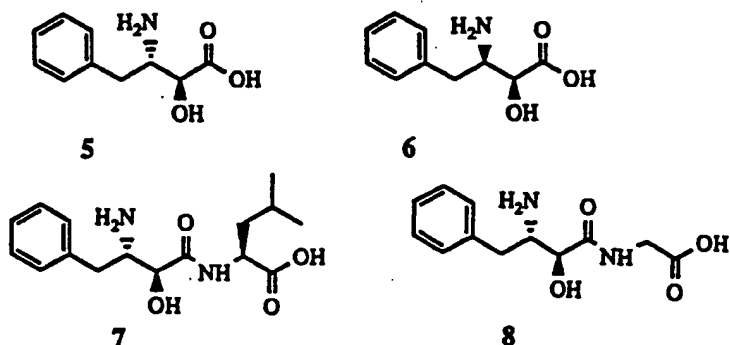
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revealed that the configuration at both 2- and 3-positions are important for inhibitory activity.

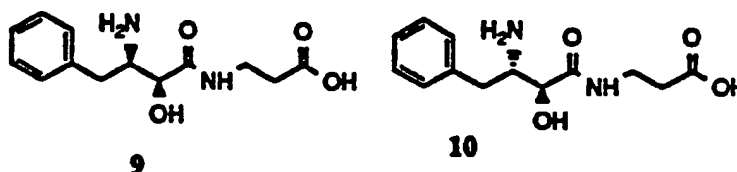
The isomer (2S,3S)-AHPA methyl ester, Compound 2, was the most potent with an inhibition constant $K_i = 50 \mu\text{M}$. The other three isomers, (2R,3S), (2S,3R) and (2R,3R)-AHPA methyl ester Compounds 1, 3 and 4, exhibited poor or no inhibition.

A free amino group was necessary as N-Boc-(2S,3S)-AHPA methyl ester showed no inhibition activity. Hydrolysis of the methyl ester to free acids (see Compounds 5 and 6) resulted in a loss of their inhibition potency. The inhibition activity was improved in the case where the C-terminus of (2S,3S)-AHPA was coupled to L-leucine or glycine ($\text{IC}_{50} = 20 \mu\text{M}$ for Compound 7 and $15 \mu\text{M}$ for Compound 8), but became worse when coupled to β -alanine (Compound 10, $\text{IC}_{50} = 80 \mu\text{M}$).



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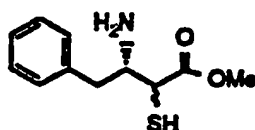
Although (2S,3R)-AHPA methyl ester, Compound 3, exhibited no activity to this enzyme, its amide derivative of L-leucine (Bestatin) is a potent inhibitor ($IC_{50} = 4 \mu M$). Orning, J. Biol. Chem., 266:1375 (1991). The β -alanine derivative of Compound 2 (Compound 9), however, is a weaker inhibitor ($IC_{50} = 100 \mu M$) than Bestatin, indicating the possible involvement of another moiety in binding. Coupling of Compound 2 with D-leucine did not improve the inhibitory potency.



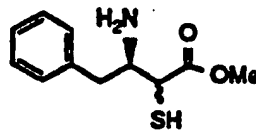
It is thought that the mode of inhibition of these AHPA derivatives is similar to that of aminopeptidase with Bestatin where the free amine and the OH group coordinate to the zinc ion. Burley, et al., Proc. Natl. Acad. Sci., 88:6916 (1991); Bartlett et al., Science, 235:569 (1987); Tronrud et al., Science, 235:571 (1987); Christianson et al., Acc. Chem. Res., 22:62 (1989); Breslow et al., Proc. Natl. Acad. Sci., 80:4585 (1983); Izquierdo-Martin et al., J. Am. Chem. Soc., 114:325 (1992); Nishizawa et al., J. Med. chem., 20:510 (1977). The AHPA derivative OH group was therefore replaced with the SH group because thiol is a better ligand for Zn^{++} . Surprisingly, the two thiol Compounds 11 and 12 were poor inhibitors, similar to the results observed in the study of aminopeptidase. Ocain et al., J. Med. chem., 31:2193 (1988).

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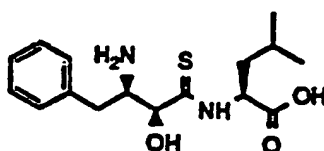


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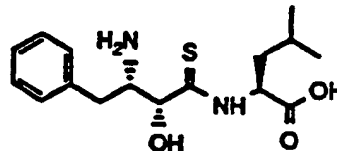
10 The thioamide analogs, Compounds 13-15, were also poor inhibitors. Phosphoramidate Compound 16 was synthesized and tested as a transition-state analog inhibitor. That compound was, however, similar to the case of

15 aminopeptidase, [Giannousis et al., J. Med. Chem., 30:1603 (1987)], a much weaker inhibitor than Compound 8. The fluoroketone Compounds 17 and 18 did not inhibit the enzyme at 1 mM concentration.

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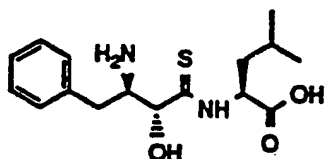


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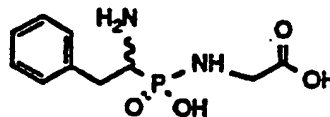


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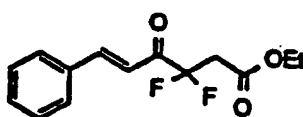


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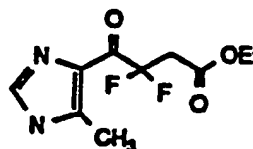


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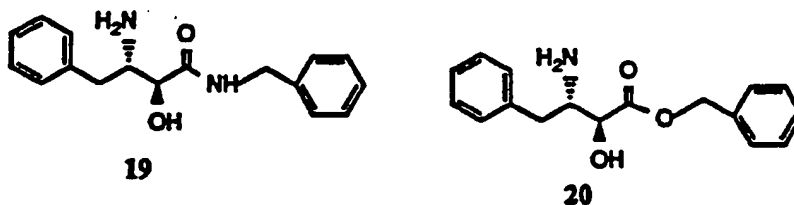
After considering the natural substrate structure and the mechanism of aminopeptidase inhibition, [Burley, et al., Proc. Natl. Acad. Sci.,

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88:6916 (1991); Bartlett et al., Science, 235:569
(1987); Tronrud et al., Science, 235:571 (1987);
Christianson et al., Acc. Chem. Res., 22:62 (1989);
Breslow et al., Proc. Natl. Acad. Sci., 80:4585 (1983);
5 Izquierdo-Martin et al., J. Am. Chem. Soc., 114:325
(1992); Nishizawa et al., J. Med. Chem., 20:510 (1977)],
another class of compounds (including Compounds 21, 22,
25 through 30, 44 and 45) were prepared, that are better
inhibitors than those simply based on the amidase
10 activity. These inhibitors contain a transition-state
mimic of the enzyme-catalyzed amide cleavage as a "core"
and additional complementarity components (the at least
two phenyl ring aromatic moieties) that resemble the
hydrophobic conjugated polyene system of the natural
15 substrate LTA₄ that binds to the enzyme more tightly
than do the amide substrates.

An α -keto ester is preferred over an α -keto
amide. [α -Keto amide inhibitors of aminopeptidase were
reported by Ocain et al., J. Med. Chem., 35:451 (1992).
20 For other types of keto ester inhibitors, see Hori et
al., in Peptides; Structure and Function, Proceedings of
the 9th American Peptide Symposium; Deber et al., eds.,
Pierce Chemical Co., Rockford, IL. 1985 pp.819;
Angelaastroc et al., J. Med. Chem., 33:11 (1990); Hu et
25 al., Arch. Biochem. Biophys., 281:271 (1990)]. The
reason for this preference is based on the inhibition
results of Compounds 19 and 20, where the ester
derivative bound more tightly than the amide.

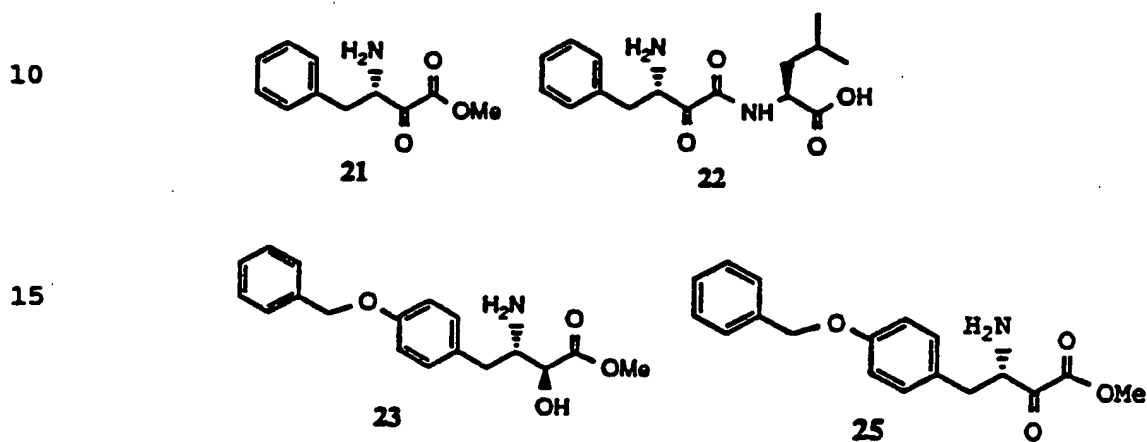
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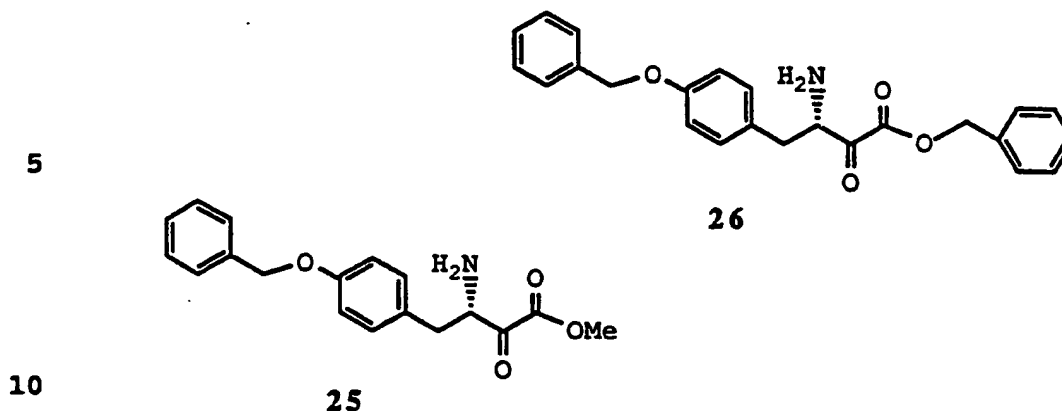
The α -keto amide with a free carboxyl group (Compound 22) was, however, a good inhibitor ($IC_{50} = 0.5 \mu M$) and the inhibition potency was better than the corresponding α -(S)-OH derivative Compound 7.

5 Interestingly, although Compound 21 is comparable with Compound 2, Compound 25 ($IC_{50} = 0.6 \mu M$) bound to the enzyme 30 times more tightly than did Compound 23.



Further adjustment of the inhibitor structure at P1' and P1-P3 sites led to the development of a β -amino- α -keto ester containing three phenyl rings, Compound 26, with $IC_{50} = 0.2 \mu M$ and $K_i = 0.046 \mu M$ (46 nM). Both 1H -NMR and ^{13}C -NMR studies indicate the β -amino- α -keto esters are completely hydrated, [For Compounds 25 and 26, the β -protons in their 1H -NMR's appear at about 3.9 ppm (hydrated form) in D_2O and about 5.0 ppm (ketone form) in DMSO; the α -carbons in the ^{13}C -NMR's appear at about 92 ppm (hydrated form) in 10 percent D_2O - DMSO and at about 188 ppm (ketone form) in DMSO], which suggests that the inhibitor exists as a gem-diol bound in the enzyme active site.

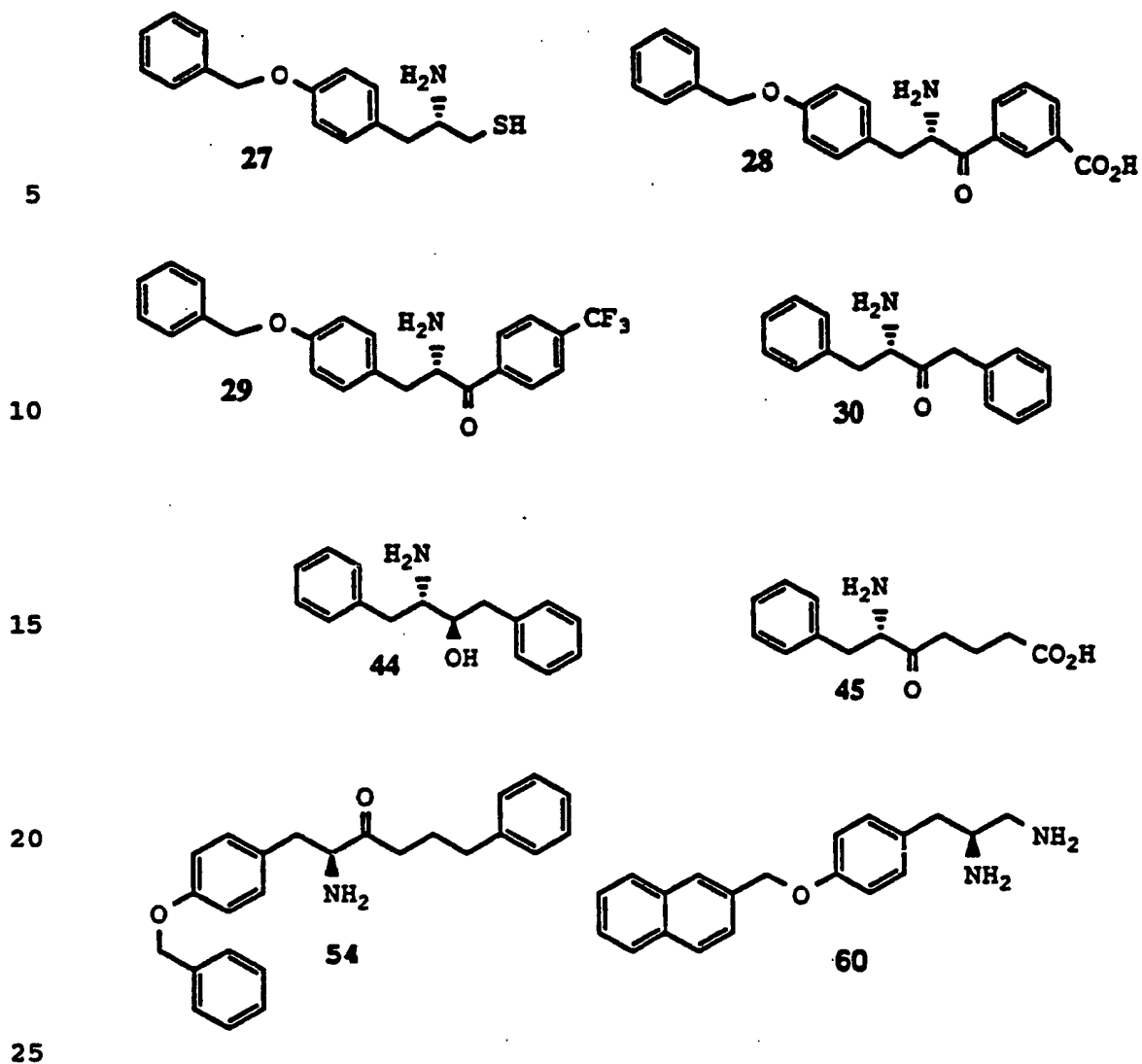
- 35 -



The free amino group and one of the hydroxyl groups may coordinate to the Zn^{++} (as N-Boc and N-Cbz derivatives are not inhibitors) and the other hydroxyl group may interact with the general base ($-CO_2^-$) via H-bonding. Unlike Bestatin and Captopril, Compounds 24, 25 and 26 are selective for LTA_4 binding. Those compounds are very weak inhibitors of other aminopeptidases; the IC_{50} values are $>100 \mu M$ for 80 μM and $>100 \mu M$ for aminopeptidase M, and 80, 50 and $>100 \mu M$ for cytosolic leucine aminopeptidase, respectively.

Attention was turned to the synthesis of more stable, non-peptide transition-state analog inhibitor Compounds 27-30, 44, 45, 54 and 60. These compounds, except 27 and 54, are, however, not as potent as 26, probably due to the lack of complete hydration of the carbonyl group.

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Compound 27 is so far the best inhibitor of LTA₄ hydrolase, with Compound 54 not being far behind. It inhibits the aminopeptidase activity with $K_i = 18$ nM and the epoxide hydrolase activity with $K_i = 0.1$ mM. It is a weaker inhibitor of aminopeptidase M and cytosolic leucine aminopeptidase with $IC_{50} = 0.5$ mM and >50 mM, respectively. Compound 27 probably forms a tight-binding complex in which the Zn^{++} coordinates to the amine and the thiol groups. Further investigation on

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the mechanisms of inhibition with Compounds 26 and 27 is in progress.

Table 2, below, provides IC_{50} or K_i values for each of Compounds 1-30, 44, 45, 54 and 60, as well as Bestatin and Captopril.

5

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Table 2
Inhibition Constants of the Listed Compounds for
LTA₄ Hydrolase^a

5	Compound	K _i	Compound	K _i
	1	IC ₅₀ >0.5 mM ^b	18	IC ₅₀ >1 mM ^d
10	2	50 μM	19	IC ₅₀ >100 μM
	3	NI ^c	20	IC ₅₀ =0.8 μM
	4	NI ^c	21	IC ₅₀ =80 μM
15	5	IC ₅₀ >1 mM	22	IC ₅₀ =0.5 μM
	6	NI ^c	23	IC ₅₀ =20 μM
20	7	IC ₅₀ =20 μM	24	IC ₅₀ =0.8 μM
	8	15 μM	25	IC ₅₀ =0.6 μM
	9	IC ₅₀ ≈100 μM	26	46 nM
25	10	IC ₅₀ ≈80 μM	27	18 nM
	11	IC ₅₀ ≈250 μM	28	IC ₅₀ =3 μM
30	12	IC ₅₀ >250 μM	29	IC ₅₀ =10 μM
	13	IC ₅₀ >0.1 mM ^d	30	14 μM
	14	IC ₅₀ >9,1 mM ^d	44	IC ₅₀ =140 μM
35	15	IC ₅₀ >0.1 mM ^d	45	IC ₅₀ =20 μM
	16	IC ₅₀ ≈200 μM	54	IC ₅₀ =30 nM
40	17	IC ₅₀ >5 mM ^d	60	IC ₅₀ =0.55 μM
			Bestatin	IC ₅₀ =4 μM (0.2 μM ^e)
			Captopril	IC ₅₀ =0.07 μM ^e
45				

^a All assays were performed in Tris-HCl buffer (0.05 M, pH 8.0) with L-alanyl p-nitroanilide (1.5 mM) as substrate unless otherwise indicated. LTA₄ hydrolase (1.4 μg) purified from human leukocytes was added for each assay (final volume = 1.0 mL). p-Nitroaniline

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formation was monitored spectrophotometrically at 405 nm. Thiol compounds were assayed in the presence of 5 mM DTT. The values shown are within ± 5 percent accuracy. Dixon plot was used to determine the K_i values.

5

^b Less than 50 percent inhibition was observed at this concentration.

10

^c NI, no inhibition observed with 1 mM inhibitor in the assay.

^d Less than 10 percent inhibition was observed at this concentration.

15

^e Measured against L-lysine p-nitroanilide. Orning et al., J. Biol. Chem., 266:1375 (1991).

20

Several Further assays were conducted that illustrate the efficacy of a contemplated compound.

25

For the results shown in Tables 3 and 4, below, compounds were dissolved in DMSO and then diluted in Hank's BSS. Blood drawn from a human volunteer, and a compound solution at one of three concentrations (Table 3) or a single concentration (Table 4), or solution alone (vehicle) was added to a one ml aliquot of the blood. After incubation for 15 minutes, 14 μ g of calcium ionophore A-23187 (as the free acid) was added, and the incubation continued for another 15 minutes.

30

The reactions were stopped by immersion of the containers into slush ice, and the samples were centrifuged at 11,000 xg to pellet red cells and leukocytes. An aliquot of the plasma was then assayed for the presence of thromboxane B_2 or leukotriene B_4 by standard radioimmunoassay techniques. The data in Table 3 and 4 are expressed as percent inhibition of eicosanoid produced in compound-treated blood compared to vehicle-treated blood.

40

- 40 -

Table 3
Inhibition of Thromboxane B₂ (TXB₂) and
Leukotriene B₄ (LTB₄)

5	<u>Compound</u> <u>Number</u>	<u>Concentration</u> <u>(μM)</u>	<u>% Inhibition</u> <u>TXB₂</u>	<u>% Inhibition</u> <u>LTB₄</u>
	26	10	28	24
		3	7	17
10		1	8	15
	24	10	4	39
		3	17	44
		1	0	7
15	25	10	41	39
		3	20	59
		1	25	29
20	10	10	19	15
		3	21	39
		1	12	14
	7	10	24	37
25		3	15	10
		1	21	25

As can be seen, the compounds assayed exhibited similar potencies. Tepoxalin, which inhibits cyclooxygenase production of PGD₂ (IC₅₀ 0.2 μ M) and 5-lipoxygenase production of 5-HETE (IC₅₀=1.29 μ M), is more potent than any compound in this table.

35

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Table 4

	<u>Compound Number</u>	<u>Concentration (μM)</u>	<u>% Inhibition TXB₂</u>	<u>% Inhibition LTB₄</u>
5	45	10	12	11
	30	10	6	15
10	7	10	0	25
	29	10	0	10
	24	10	0	18
15	21	10	0	8
	28	10	0	16
20	27a	10	24	100
	27	10	100	100

25 The data of Tables 3 and 4 were taken at different times and illustrate differences in absolute percentages of inhibition for the assays as can be seen for the results for Compounds 10 and 7 in both tables. The complete inhibitions exhibited by Compounds 27 and 27a were startling.

30 IC₅₀ Values were calculated for Compounds 27 and 27a in this assay using concentrations of about 0.3-10 μ M. Those values were found to be about 0.81 μ M for Compound 27a and about 1.1 μ M for Compound 27.

35 Another set of assays similar to those of Table 2, but also including studies of epoxide hydrolase activity of LT₄ hydrolase were also conducted. The data from those studies are provided in Table 5, below. The data were obtained as discussed hereinafter.

40

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Table 5Inhibition of Epoxide Hydrolase Activity

5	<u>Compound Number</u>	<u>IC₅₀ Epoxide Hydrolase (μM)</u>
	7	100
10	8	NI
	20	100
	24	2.2
15	25	100
	28	NI
20	29	>100
	27	0.2
	30	>100
25	Captopril	240
	Bestatin	0.3
30	<hr/>	

NI = No inhibition at 100 μM concentration.

35 LTA₄ hydrolase used for Table 5 results was
 obtained from human leukocytes and was purified to
 apparent homogeneity by streptomycine and ammonium
 sulfate precipitations, ion-exchange, hydrophobic
 interaction and chromatofocusing chromatographies, as
 40 described in Wetterholm et al., Biochim. Biophys. Acta,
1080:96-102 (1991). After the final purification step,
 the buffer was changed to 10 mM Tris-Cl, pH 8 by
 repeated centrifugation on a Centricon-30
 microconcentrator (Amicon, Danvers, MA), lyophilized and
 45 stored at -20°C. SDS-PAGE was performed on a Phast
 system (Pharmacia) using 10-15 percent gradient gels.

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Bands of protein were visualized by staining with coomassie brilliant blue. Protein concentrations were determined by the method of Bradford, Anal. Biochem., 72:248-254 (1976), using bovine serum albumin as standard.

5 The inhibition of LTA₄ hydrolase activity was determined by incubating purified enzyme (2.5 µg in 100 µl of 50 mM HEPES buffer, pH 8) for 30 minutes at room temperature, with the respective inhibitor dissolved in
10 a mixture of DMF/H₂O (80/20, v/v). Compound 27 was dissolved in 90 percent methanol containing 10-5 mM DTT. LTA₄ (3 nmol) was added in 0.5 µl of THF and the reaction was permitted to proceed for 15 second prior to quenching with 200 µl methanol.

15 A defined amount of internal standard, prostaglantin B₁ (PGB₁; Upjohn) was added, the samples were acidified to pH 3 with 0.1 M HCl, and subjected to solid phase extraction using Chromabon C₁₈ columns, as described in Steinhilber et al., J. Chromatogr.,
20 493:305-400 (1991). For RP-HPLC, a column (Nova-Pak C₁₈, 4 µm, Radial-Pak cartridge, 5 X 100 mm; Waters) was eluted with a mixture of methanol/water/acetic acid (70/30/0.01; v/v/v) at a flow rate of 1.2 ml/minute. The absorbance of the eluate was monitored continuously
25 at 270 nm. Quantitations of LTB₄ were made from area integration using Baseline 810 computer software (Waters) based on a standard curve obtained from analysis of known amounts of the respective compounds.

Compounds 24 and 27 were also studied for
30 their abilities to inhibit 5- and 15-lipoxygenase using calcium ionophore A-23187 stimulated polymorphonuclear leukocytes (PMNL) in the presence of exogenous arachidonic acid. 5-Lipoxygenase activity (production of 5-HETE) was inhibited, at 1-2 log orders higher than
35 those for inhibition of LTA₄ hydrolase activity. No

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inhibition of 15-lipoxygenase activity was noted. LTC₄ Synthase activity in human platelets was also not inhibited. LTB₄ Formation in calcium ionophore A-23187-stimulated intact PMNL was dose dependently inhibited by
5 Compounds 24 and 27 with IC₅₀ values of 100-200 nM for both substances. Thus, Compound 24 was much more active in this assay using intact PMNL than in assays with the isolated enzyme.

The above results indicate the specificity of
10 the contemplated compounds towards LTA₄ as compared to other closely related enzymes in the arachidonic acid cascade. Most prior attempts to control leukotrienes have focused on 5-lipoxygenase, which converts
15 arachidonic acid into LTA₄, the common substrate for both LTA₄ hydrolase and LTC₄ synthase. The selectivity of these compounds towards only LTA₄ hydrolase, which catalyzes the final and rate-limiting step in LTB₄ biosynthesis can be beneficial in reducing drug side effects.

20

Best Mode for Carrying out the Invention

General Methods.

¹H-NMR spectra were obtained at 300, 400 or 500 MHz, ¹³C-NMR spectra were obtained at 100 or 125 MHz.
25 All chemical shifts are reported in δ units (ppm) relative to tetramethylsilane (assigned to 0.0 ppm). Thin-layer chromatography (TLC) was performed on silica gel plates (0.25 mm, Merck) by using the following detection methods: UV, visualized under an ultraviolet
30 lamp; I₂ on silica; AMA, dipped into a solution containing 5 percent ammonium molybdate, 4.2 percent H₂SO₄ and 0.6 percent sodium arsenate and heated on a hot plate. Flash chromatography was performed with silica gel (230-400 mesh, Merck). The following

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solvents were used: chloroform (C), ethyl acetate (EA), hexane (H), methanol (M or MeOH), ethanol (EtOH).

5 Example 1: Method A: General Procedure for Peptide-Bond Formation

 The N-deprotected amino acid (1 equivalent) was dissolved to a concentration of 0.1 M in DMF, and a C-protected compound (1 equivalent, usually as a HCl salt) and HOBt (1.5-2 equivalent) were added, followed
10 by N-methylmorpholine (1 equivalent) and DCC (1 equivalent). The mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate and the solid was removed by filtration. The filtrate was washed with 10 percent citric acid
15 (2x), saturated sodium bicarbonate (2x), and brine. The organic layer was dried over MgSO₄ and concentrated to dryness in vacuo. The final product was either directly used in the next step or purified by flash
20 chromatography depending on the purity as judged by NMR spectra or TLC.

 Example 2: Method B: General Deprotection Procedure

 (1) The protected peptide was dissolved to a concentration of approximately 0.5 M in 50 percent
25 trifluoroacetic acid in dry dichloromethane and the mixture was stirred at room temperature for three hours. The solvent was removed in vacuo and the remaining trifluoroacetic acid was removed by repeated evaporation from dichloromethane in vacuo.

30 (2) The protected peptide was dissolved to a concentration of approximately 0.2-0.5 M in ether and dry HCl (gas) was bubbled in until saturated. The reaction was stirred at room temperature overnight (about 18 hours). The solid was triturated with ether
35 and collected by filtration.

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(3) The methyl ester was dissolved in 20 percent methanol in THF to a concentration of about 0.1 M and 1.1 equivalents of lithium hydroxide dissolved in 0.5-1 mL water were added. The reaction progress was monitored by TLC. The reaction took about one to three hours to complete. Most solvents were removed in vacuo and the residue was dissolved in ethyl acetate. The organic layer was washed with 10 percent citric acid and then water, dried over MgSO_4 . The free acid was obtained after removal of the solvent in vacuo.

Example 3: Method C: Preparation of N-t-Butyloxycarbonyl-AHPA (3-Amino-2-Hydroxy-4-Phenylbutanoic Acid) Methyl Ester (Scheme 1)

To a stirred solution of L-phenylalanine methyl ester (10.8 g, 50 mmol, hydrochloride salt) in 150 mL of 20 percent THF in water was added sodium bicarbonate (8.4 g, 100 mmol) at room temperature, followed by di-t-butyl dicarbonate (10.9 g, 50 mmol). The mixture was stirred at room temperature overnight (about 18 hours). The reaction mixture was extracted with ether (2 x 100 mL), and the ether layers were combined and dried over MgSO_4 . Boc-phenylalanine methyl ester was obtained as a pale yellow oil. The oil was dissolved in 70 mL dry toluene and cooled to -77°C under nitrogen and 125 mmol diisobutylaluminum hydride (1.0 M in hexane, 125 mL, 2.25 equivalent) were added in 15 minutes. Methanol (10 mL) was added immediately to quench the reaction. The reaction mixture was poured to a solution of 100 mL of 20 percent sodium potassium tartrate (Rochelle salt) and stirred at room temperature until the two layers separated. The organic layer was taken and concentrated in vacuo.

The resulting aldehyde was treated with a solution of sodium metabisulfite (14.5 g, 75 mmol) in 50

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mL water and then to the emulsion was added a solution of potassium cyanide (4.9 g, 75 mmol) in 50 mL water with stirring. The reaction was stirred at room temperature for about five hours and the cyanohydrin was extracted twice with ether and the ether layer was combined and concentrated in vacuo to dryness. The residue was treated with 25 percent hydrochloric acid and the solution was stirred at 80°C for 12 hours.

5 The water was removed in vacuo to give crude (2RS,3S)-AHPA as a brown solid, which was dissolved in 100 mL of 20 percent THF in water and the pH was adjusted to about 8 by addition of 10 N NaOH solution. To the solution was added sodium carbonate (8.3 g, 100 mmol) and di-*t*-butyl dicarbonate (21.8 g, 100 mmol). 15 The mixture was stirred at room temperature overnight and the pH of the reaction was adjusted to 2 with 6 N HCl and extracted with ethyl acetate. The organic layer was taken and washed with 10 percent citric acid once. The product, *N*-*t*-butyloxycarbonyl-AHPA, was again 20 extracted by 1N NaOH to the aqueous phase. The side products and other impurities were left in the organic layer. The aqueous phase was acidified to pH 2 and extracted with ethyl acetate. The ethyl acetate was removed and *N*-Boc-AHPA was obtained as pale yellow 25 solid.

The solid was dissolved in 15 mL DMF, and methylated by adding potassium bicarbonate (8 g, 80 mmol) and iodomethane (11.4 g, 80 mmol). After 12 hours, the reaction was diluted with ethyl acetate and washed with 10 percent citric acid twice and then water once. Concentration of the organic layer afforded final product (8.5 g, 55 percent overall yield) as a mixture of two diastereomers (2R:2S ~ 7:3), which were separated by preparative TLC (2mm, Merck about 200 mg each) by 35 using 35 percent EA in H as solvent or by flash column

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(5 percent EA in H, 10 percent EA in H then 20 percent EA in H).

5 N-Boc-(2R,3S)-AHPA methyl ester (Compound 32R):
TLC (R_f 0.33, 25 percent EA in H, UV or AMA); $^1\text{H-NMR}$
(CDCl_3 , 300 MHz) δ 1.39 (s, 9H), 2.9 (m, 2H), 3.75 (s, 3H), 4.05 (d, $J=4.0$ Hz, 1H), 4.25 (m, 1H), 4.78 (d, $J=9.0$ Hz, 1H), 7.15-7.35 (m, 5H); $[\alpha]_D^{24} = -78.5^\circ$ ($c = 1.0$, CHCl_3).

10 N-Boc-(2S,3S)-AHPA methyl ester (Compound 32S):
TLC (R_f 0.21, 25 percent EA in H, UV or AMA); $^1\text{H-NMR}$
(CDCl_3 , 300 MHz) δ 1.39 (s, 9H), 2.8 (m, 2H), 3.75 (s, 3H), 4.33 (m, two overlapped protons), 4.85 (d, $J=8.0$ Hz, 1H), 7.15-7.35 (m, 5H); $[\alpha]_D^{24} = -15.5$ ($c = 1.0$, CHCl_3).

15 Example 4: (2R,3S), (2S,3S), (2S,3R), (2R,3R)-AHPA
Methyl Esters (Compounds 1, 2, 3, 4)

The four isomers were made in the same way as free bases. A representative procedure is given below.
20 100 mg N-Boc-(2S,3S)-AHPA methyl ester was deprotected by the general deprotecting procedure B(1). After removal of solvent, the residue was treated with 10 mL of saturated sodium bicarbonate solution and extracted with ethyl acetate (2 x 100 mL). The organic layers
25 were combined with dried over MgSO_4 . The product was obtained as a pale yellow oil (solidified at room temperature in a few days) after removal of the solvent (60 mg, 90 percent).

30 (2S,3S)-APHA methyl ester (Compound 2): $^1\text{H-NMR}$
(CDCl_3 , 300 MHz) δ 2.58 (m, 1H), 2.80 (m, 1H), 3.48 (m, 1H), 3.80 (s, 3H), 4.24 (d, $J=3.0$ Hz, 1H), 7.15-7.35 (m, 5H); $[\alpha]_D^{24} = -3.3^\circ$ ($c=1.2$, 1N HCl).

(2S,3R)-AHPA methyl ester (Compound 3): $^1\text{H-NMR}$
(CDCl_3 , 300 MHz) δ 2.74 (m, 1H), 2.92 (m, 1H), 3.35 (m,

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1H), 3.80 (s, 3H), 4.08 (d, J=3.5 Hz, 1H), 7.2-7.35 (m, 5H); $[\alpha]_D^{24} = +19.6^\circ$ (c=0.84, 1 N HCl).

Example 5: (2S,3S) and (2S,3R)-AHPA (Compounds 5, 6)

5 The two compounds were made in the same manner as trifluoroacetic acid salt. N-Boc-(2S,3S)-AHPA methyl ester (200 mg, 0.65 mmol) was demethylated according to Method B(3) to give the corresponding free acid. The acid was deprotected by following Method B(1) to give
10 (2S,3S)-AHPA as a trifluoroacetic acid salt (160 mg, 80 percent):

 (2S,3S)-AHPA $^1\text{H-NMR}$ (D_2O , 400 MHz) δ 2.98 (m, 2H), 4.0 (m, 1H), 4.50 (d, J=3.0 Hz, 1H), 7.20-7.40 (m, 5H); $[\alpha]_D^{24} = -2.2^\circ$ (c=0.67, 1N HCl).

15 (2S,3R)-AHPA: $^1\text{H-NMR}$ (D_2O , 300 MHz) δ 3.0 (m, 2H), 3.88 (m, 1H), 4.25 (d, J=3.0 Hz, 1H), 7.20-7.40 (m, 5H). $[\alpha]_D^{24} = +11.8^\circ$ (c=1.02, 1N HCl).

Example 6:

20 The following compounds were prepared by coupling appropriate N-Boc acids and amino acid t-butyl esters according to Method A and deprotected by following Method B(1).

25 A: (2S,3S)-AHPA-L-Leu (Compound 7) [Nishizawa et al., J. Med. Chem., 20:510 (1977)] (trifluoroacetic acid salt): $^1\text{H-NMR}$ (D_2O , 400 MHz) δ 0.90 (m, 6H), 1.6-1.8 (m, 3H), 2.95 (d, J=13 Hz, 2H), 4.02 (m, 1H), 4.25 (m, 1H), 4.56 (d, J=3.0 Hz, 1H), 7.25-7.40 (m, 5H); $[\alpha]_D^{24} = -26.5^\circ$ (c=1.0, H_2O).

30 B: (2S,3S)-AHPA-Gly (Compound 8) (trifluoroacetic acid salt): $^1\text{H-NMR}$ (D_2O , 400 MHz) δ 3.0 (m, 2H), 3.89 (s, 2H), 4.02 (m, 1H), 4.55 (d, J=3.0 Hz, 1H), 7.15-7.35 (m, 5H); $[\alpha]_D^{24} = -19.0^\circ$ (c=0.1, H_2O) HRMS: 253.1183 (M+H) $^+$, calcd. for ($\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_4\text{+H}$) 253.1188.

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C: (2S,3S)-AHPA- β -Ala (Compound 10)
(trifluoroacetic acid salt): $^1\text{H-NMR}$ (D_2O , 300 MHz) δ
2.45 (t, $J=7.8$ Hz, 2H), 2.88 (m, 2H), 3.25 (t, $J=8.0$ Hz,
2H), 3.95 (m, 1H), 4.38 (d, $J=3.0$ Hz, 1H), 7.2-7.4 (m,
5H); $[\alpha]_D^{25} = -16.7^\circ$ ($c=1.02$, H_2O) HRMS: 267.1345 ($\text{M}+1$) $^+$,
calcd for ($\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_4+\text{H}$) 267.1345.

Example 7: Endothiodipeptides (Compounds 13 and 15)

Boc-(2S,3R)-AHPA methyl ester (500 mg, 1.6 mmol)
was dissolved in a solution of 2,2-dimethoxypropane (15
mL) and *p*-toluenesulfonic acid (100 mg, dried
azeotropically from benzene). After stirring at room
temperature for 36 hours, the reaction was diluted with
ethyl acetate and the organic layer was washed with
saturated NaHCO_3 (2x) and water, concentrated in vacuo
to dryness. Purification of the residue on silica gel
(5 percent EA, 45 percent H in C, then 10 percent EA in
H) afforded the isopropylidene methyl ester as a pale
yellow oil which was hydrolyzed by following the general
deprotection procedure to afford an acid as a white
solid (470 mg, 88 percent for two steps). The general
procedure for peptide formation was followed to effect
the coupling of the acid (425 mg, 1.27 mmol) and
L-leucine *t*-butyl ester (HCl salt, 310 mg, 1.5 mmol),
giving a product as a white solid (505 mg, 79 percent)
after purification on silica gel (5 percent EA, 20
percent H in C).

The protected dipeptide product (150 mg, 0.3
mmol) was dissolved in 7 mL dry benzene and the
Lawesson's reagent (75 mg, 0.18 mmol, Aldrich) was
added. The resulting solution was stirred at 88°C (bath
temperature) for 1.5 hours in a capped flask. TLC
showed a complete reaction (starting material $R_f = 0.61$,
product $R_f = 0.71$, 25 percent ethyl acetate in hexane,
UV or AMA). The reaction was concentrated in vacuo and

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the residue was purified on preparative TLC (2 mm, Merck, 30 percent EA in H) to afford the protected endothiodipeptide as a pale yellow oil (150 mg, 96 percent); MS: 512 (M+1)⁺. The thio compound was treated with 10 mL trifluoroacetic acid and stirred at room temperature for five hours, then 2 mL methanol were added to the solution and stirred at 70°C (bath temperature) for 6 hours. The solvent was removed in vacuo and the residue lyophilized from benzene to give product Compound 13 as a white solid (75 mg, 77 percent). NMR and TLC showed the existence of other diastereomer Compound 15, perhaps the racemization took place at the carbon α to thioamide during the thionation. The product (35 mg) was further purified by silica gel preparative TLC (2 mm, Merck, 30 percent methanol in chloroform, developed twice) to give pure Compounds 13 (20 mg) and 15 (8 mg).

Compound 13 (TFA salt): TLC (R_f 0.36, 25 percent M in C, UV); ¹H-NMR (400 MHz, D₂O) δ 0.85 (d, 3H, J=6.4 Hz), 0.88 (d, 3H, J=6.4 Hz), 1.60-1.89 (m, 3H), 2.78 (m, 1H), 2.97 (m, 1H), 3.82 (m, 1H), 4.35 (d, 1H, J=3.0 Hz), 4.60 (m, 1H), 7.23-7.38 (m, 5H); [a]_D²² = -17.2° (c=0.87, 1H HCl); MS: 325 (M+1)⁺.

Compound 15 (TFA salt): TLC (R_f 0.28, 25 percent M in C, UV); ¹H-NMR (400 MHz, D₂O) δ 0.90 (d, 3H, J=6.4 Hz), 0.94 (d, 3H, J=6.4 Hz), 1.66-1.89 (m, 3H), 2.62 (m, 1H), 2.94 (m, 1H), 3.91 (m, 1H), 4.62 (d, 1H, J=3.0 Hz), 4.68 (m, 1H), 7.26-7.38 (m, 5H); [a]_D²² = +15.6° (c=0.15, 1H HCl), MS: 325 (M+1)⁺.

Example 8: Endothiodipeptide (Compound 14)

The procedure for the preparation of Compound 14 was the same as that for the preparation of Compound 13: TLC (R_f 0.42, 25 percent M in C, UV or AMA); ¹H-NMR (400 MHz, D₂O) δ 0.81 (d, 3H, J=6.5 Hz), 0.86 (d, 3H, J=6.5

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Hz), 1.57 (m, 1H), 1.65 (m, 1H), 1.69 (m, 1), 2.88 (m, 2H), 3.81 (m, 1H), 4.29 (d, 1H, J=2.4 Hz), 4.65 (m, 1H), 7.20-7.35 (m, 5H); $[\alpha]_D^{22} = +11.1^\circ$ (c=0.57, 1N HCl).

5 **Example 9: Preparation of Compound 18**

(a): -Triphenylmethyl-4(5)-methylimidazole-5(4)-carboxylaldehyde

10 4-Methyl-5-imidazolemethanol hydrochloride (1.5 g, 10 mmol, Aldrich) was dissolved in a solution of triethylamine (2.8 mL, 20 mmol), DMF (10 mL) and dichloromethane (20 mL) at room temperature, triphenylmethyl chloride (2.76 g, 9.9 mmol) was added.
15 The resulting solution was stirred at room temperature overnight and solid came out the solution. The solid was collected by filtration and washed with H₂O and ethyl acetate, air-dried to give the protected imidazole alcohol as two isomers (3.1 g, 88 percent).
20 The protected imidazole alcohol (1.5 g, 4.2 mmol) was suspended in 30 mL dry 1,4-dioxane and the solution was heated with a hair dryer to dissolved most of the solid. Activated MnO₂ (1.5 g, 4 x 4.2 mmol, Aldrich) was added and the reaction was stirred at room temperature for 24
25 hours. The dark reaction solution was filtered through a Celite pad and the filtrate was concentrated in vacuo to dryness. The residue was purified on silica gel (5 percent EA, 5 percent H in C) to give the title compound as a white solid (1.0 g, 67 percent): TLC (R_f 0.5, 50
30 percent EA in H). ¹H-NMR (300 MHz, CDCl₃) δ 1.85 (s, 3H, -CH₃), 9.9 (s, 1H, -CHO), MS: 353 (M+H)⁺.

(b) Difluoroimidazolealcohol Analogue

The aldehyde was coupled with ethyl bromodifluoroacetate according to a published procedure.
35 [Thaisrivongs et al., J. Med. Chem., 29:2080 (1986).] Activated zinc (262 mg; 64.0 mmol) and 10 mL THF in a

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two-neck flask was refluxed at 75-80°C and 0.52 mL (4.0 mmol) ethyl bromodifluoroacetate were added through a syringe continuously over 10 seconds. The aldehyde (0.71 g, 2.0 mmol) dissolved in 5 mL CH₂CL₂ and 5 mL THF was added over one minute. The resulting solution was refluxed for 15 minutes. The solvent was removed in vacuo and the residue dissolved in chloroform and the chloroform layer was extracted with 5 percent EDTA solution (pH = 10) twice, dried over MgSO₄. Removal of chloroform gave the crude product as a white solid (0.8 g, 84 percent). The compound was used in the next step without further purification: TLC (R_f 0.62, 10 percent M in C, UV or AMA).

(c) Difluoroketoneimidazole Analogue, Compound 18

The oxidation procedure was described before. [Pfitzner et al., J. Am. Chem. Soc., 87:5561 (1965).] The crude alcohol (200 mg, 0.42 mmol) was dissolved in dry benzene (3 mL) and DMSO (0.15 mL, 5 x 0.42 mmol), DCC (435 mg, 5 x 0.42 mmol) and anhydrous phosphoric acid (25 mg, 0.25 mmol) were added. The mixture was stirred at room temperature for 24 hours. The solid was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified on silica gel preparative TLC (2 mm, Merck, 30 percent ethyl acetate in hexane) to give the triethyl-protected difluoroketone as a white solid (105 mg, 53 percent): TLC (R_f's 0.35 and 0.30, two isomers, 25 percent ethyl acetate in hexane, UV or AMA). Deprotection was done by dissolving the compound in 5 mL trifluoroacetic acid and stirring for four hours at room temperature. The acid was removed in vacuo and the residue was dissolved in 4 mL H₂O (2 x 2 mL), filtered to remove solid. The filtrate was lyophilized to give the final product 18 as a white solid (42 mg, 82 percent). ¹H-NMR (300 MHz, D₂O, hydrated and ketone forms) δ 1.20 (two t, 3H, J=7.2 Hz),

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2.33 and 2.52 (two s, 3H, -CH₃), 4.29 (two q, 2H, J=7.2 Hz), 8.53 and 8.54 (two s, 1H, aromatic H); MS (methanol as solvent), 233 (M+1)⁺, 251 (M+18+H)⁺, hydrated form (265 (M+32+H)⁺, hemiketal with methanol.

5

Example 10: (2S,3S)-AHPA Benzyl Amide (Compound 19)

N-Boc-(2S,3S)-AHPA (90 mg, 0.31 mmol) was coupled with benzyl amine (70 mg, 0.65 mmol) according to the Method A and deprotected by following Method B(1) to give the title compound as a white solid (101 mg, overall 82 percent in two steps, trifluoroacetic acid salt). ¹H-NMR (DMSO, 300 MHz) δ 2.78 (d, J=6.8 Hz, 2H), 3.68 (m, 1H), 4.20 (d, J=8.1 Hz, 2H), 4.28 (d, J=3 Hz, 1H), 7.15-7.35 (m, 5H); MS: 285 (M+1)⁺; [α]_D²⁶ = -24.8° (c=0.70, AcOH).

15

Example 11: Method D: General Procedure for the Preparation of Phenol Benzyl Ether or Benzyl Ester (Scheme 2)

20 The following preparation is typical.

(2R,3S)-3-N-t-Butyloxycarbonylamino-2-Hydroxy-4-(4-benzyloxyphenyl)-Butanoic Acid, methyl ester (Compound 34S) and its diastereomer (Compound 34R). Compound 33 (prepared from Boc-Tyr(benzyl) methyl ester according to Method C) (1.2 g, 3.7 mmol) was dissolved in 5 mL of dry DMF and Cs₂CO₃ (2.4 g, 7.4 mmol, Aldrich), tetrabutylammonium iodide (50 mg, Aldrich), benzyl bromide (1.2 g, 1.8 x 3.7 mmol) were added sequentially. The disappearance of the starting material was followed by TLC (usually took two to five hours). The reaction mixture was diluted with ethyl acetate and the organic layer was washed with water (2x), concentrated in vacuo to dryness. The residue was purified on silica G (20 percent EA in H) to give (2R,3S) isomer (680 mg, 44 percent, white wax), (2S,3S)

35

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isomer (450 mg, 29 percent, white wax) and a mixture of the two (260 mg, 17 percent). (2R,3S) isomer (Compound 34R): TLC (R_f 0.58, 50 percent EA in H, UV or AMA), $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 1.38 (s, 9H), 2.84 (m, 2H), 3.73 (s, 3H), 4.05 (br s, 1H), 4.18 (m, 1H), 4.77 (d, $J=10.0$ Hz, 1H), 5.03 (s, 2H), 6.91 (d, $J=6.8$ Hz, 2H), 7.18 (d, $J=8.4$ Hz, 2H), 7.30-7.43 (m, 5H); (2S,3S) isomer (Compound 34S): TLC (R_f 0.44, 50 percent EA in H, UV or AMA); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 1.38 (s, 9H), 2.73 (m, 2H), 3.55 (s, 3H), 4.24 (br s, 1H), 4.30 (d, $J=4.8$ Hz, 1H), 4.82 (d, $J=6.8$ Hz, 2H), 5.03 (s, 2H), 6.87 (d, $J=6.4$, 2H), 7.10 (d, $J=8.4$ Hz, 2H), 7.25-7.42 (m, 5H).

Example 12: Method E: Preparation of Compound 25

DMSO (256 mg, 3.28 mmol) was added to a solution of $(\text{COCl})_2$ (211 mg, 1.64 mmol) in 5 mL dry CH_2Cl_2 at -77°C and after being stirred at this temperature for 10 minutes, Compound 34R (170 mg, 0.41 mmol) in 5 mL of dry CH_2Cl_2 was added dropwise. After stirring at -77°C for 20 minutes, the reaction mixture was treated with triethylamine (331 mg, 3.28 mmol) and the mixture was stirred at the same temperature for 10 minutes before warming up the room temperature. The reaction mixture was diluted with 20 mL of hexane and filtered to remove the salt, the filtrate was concentrated, the residue purified on silica G (25 percent EA in H) to obtain the Boc-protected Compound 25 (clear oil, 150 mg, 89 percent). The Boc group was removed by following Method B(2) to give Compound 25 as a pale yellow solid (85 mg, 67 percent, HC salt): $^1\text{H-NMR}$ (D_2O , 400 MHz) δ 2.85 (dd, $J=8.1$, 14.3 Hz, 1H), 3.00 (dd, $J=6.8$, 14.7 Hz, 1H), 3.49 (s, 3H), 3.84 (t, $J=7.04$ Hz, 1H), 5.18 (s, 2H), 7.03 (d, $J=8.6$ Hz, 2H), 7.21 (d, $J=8.6$ Hz, 2H), 7.37-7.50 (m, 5H); $^{13}\text{C-NMR}$ (10 percent D_2O in DMSO, 100 MHz) δ 32.90, 52.33, 57.59, 69.10, 91.74, 114.81, 127.62, 127.69,

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127.83, 127.90, 128.51, 130.55, 137.10, 157.16, 169.77;
high-resolution MS: 314.1386 (M+H)⁺, calcd for (C₁₈H₁₉NO₄
+ H) 314.1392.

5 Example 13: Compound 21

 The compound was prepared by oxidation of
corresponding alcohol N-Boc-(2RS,3S)-AHPA methyl ester
(32) according to Method E and deprotected according to
Method B(2) to give the title compound (HCl salt, pale
10 yellow powder): ¹H-NMR (D₂O, 400 MHz) δ 2.88 (dd, J=8.9,
14.5 Hz, 1H), 3.12 (dd, J=6.0, 14.4 Hz, 1H), 3.65 (s,
3H), 3.89 (dd, J=6.0, 8.8 Hz, 1H), 7.30-7.43 (m, 5H);
MS: 208 (M+1)⁺, 226 (M+H₂O+1)⁺ (hydrate form).

15 Example 14: Compound 22

 The title compound was prepared by oxidizing the
corresponding alcohol, N-Boc-(2S,3S)-AHPA-L-Leu-t-but
according to Method E and deprotected according to
Method B(1) to give the title compound (trifluoroacetic
20 acid salt, pale yellow powder): ¹H-NMR (D₂O, 400 MHz) δ
0.91 (m, 6H), 1.65-1.78 (m, 3H), 2.78 (m, 1H), 3.10 (m,
1H), 3.75 (m, 1H), 4.37 (m, 1H), 7.18-7.42 (m, 5H); MS
(FAB⁻): 305 (M-1)⁻.

25 Example 15: Compound 23

 The title compound was made via deprotection of
the corresponding N-Boc protected Compound (348)
according to Method B(2) (HCl salt, white powder):
¹H-NMR (D₂O, 400 MHz) δ 2.93 (m, 2H), 4.08 (br m, 1H),
30 4.57 (d, J=2.2 Hz, 1H), 5.18 (s, 2H), 7.02 (d, J=8.4 Hz,
2H), 7.21 (d, J=8.4 Hz, 2H), 7.39-7.50 (m, 5H); [α]_D²⁴ =
+3.5 (c=1.0, AcOH); HRMS: 316.1550, calcd for
(C₁₈H₂₁NO₄+H) 316.1548.

35

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Example 16: Compound 24

Compound 348 (400 mg, 0.96 mmol) was demethylated according to Method B(3) to give the corresponding free acid (380 mg, 99 percent). The free acid (150 mg) was benzylated by following Method D to give the benzyl ester Compound 358 as a white solid (150 mg, 81 percent) after purification on silica G column (30 percent EA in H). The benzyl ester (100 mg) was deprotected according to Method B(2) to give the title compound (HCl salt, white powder, 75 mg, 86 percent): ¹H-NMR (D₂O, 400 MHz) δ 2.85 (d, J=6.9 Hz, 2H), 4.05 (br m, 1H), 4.42 (d, J=11.9 Hz, 1H), 4.57 (br s, 1H), 4.71 (d, J=12.0 Hz, 1H), 5.12 (s, 2H), 6.94 (d, J=7.8 Hz, 2H), 7.10 (d, J=8.2 Hz, 2H), 7.25-7.44 (m, 10H); [α]_D²⁴ = +3.0 (c=1.0, AcOH); HRMS: 392.1858, calcd for (C₂₄H₂₅NO₄+H) 392.1861.

Example 17: Method F: Compound 26 (Scheme 2)

The benzyl ester (RS)-35 (180 mg, 0.37 mmol) was dissolved in 8 mL dry CH₂Cl₂ under argon, and to this solution was added Dess-Martin reagent [Dess et al., J. Org. Chem., 48:4155-4156 (1983); Burkhardt et al., Tetrahedron Lett., 29:3433-3436 (1988)] (400 mg, 0.94 mmol) portionwise at room temperature with stirring. After stirring at room temperature for 1.2 hours, the reaction mixture was diluted with ether (30 mL), followed by saturated NaHCO₃ (10 mL) and Na₂S₂O₃ (1.0 g in 10 mL H₂O), and stirred until two layers became clear. The organic layer was separated and washed with H₂O and dried over MgSO₄. The Boc-protected Compound 26 was obtained as a pale yellow solid after removal of solvent and lyophilization. The compound was pure as judged by TLC and ¹H-NMR and was deprotected according to Method B (2) to give Compound 26 as a white powder (HCl salt, 120 mg, 77 percent for two steps): ¹H-NMR (20

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percent D₂O in DMSO, 400 MHz), δ 2.71 (d, J=6.9 Hz, 2H), 3.49 (t, J=6.8 Hz, 1H) 4.67 (d, J=12 Hz, 1H), 4.86 (d, J=12 Hz, 1H), 5.03 (s, 2H), 6.86 (d, J=8.4 Hz, 2H), 7.01 (d, J=8.8 Hz, 2H), 7.25-7.38 (m, 10H); ¹³C-NMR (20
5 percent D₂O in DMSO, 100 MHz) δ 32.85, 57.65, 66.97, 69.12, 91.77, 114.84, 114.81, 127.57, 127.63, 127.71, 127.92, 128.30, 128.47, 128.53, 130.14, 130.52, 135.00, 137.02, 157.19, 169.23; HRMS: 390.1709 (M+H)⁺, calcd for (C₂₄H₂₃NO₄+H) 390.1705.

10

Example 18: Compound 27

The title compound was prepared first as a Boc-protected disulfide dimer by following the same procedures as described [Fournie-Zaluski et al., J. Med. Chem., 35:1259-1266 (1992)] and then deprotected
15 according to the Method B (2) to give the compound as a white solid HCl salt): ¹H-NMR (d₄-MeOH, 400 MHz), δ 2.80 (m, 4H), 2.89 (m, 2H), 3.02 (m, 2H), 3.77 (br s, 2H), 5.06 (s, 4H), 6.99 (d, J=8.6 Hz, 4H), 7.16 (d, J=8.6 Hz, 4H), 7.32-7.45 (m, 10H), HRMS: 545.2305, calcd for (C₃₂H₃₆N₂O₂S₂+H) 545.2296.

20

Example 19: Method G: Compound 28 (Scheme 3)

(A) Compound 37

25 THP-protected 3-bromobenzylalcohol (2.71 g, 10 mmol) was added to a suspension of Mg turnings (270 mg, 11 mmol) in 20 mL dry THF under argon. The resulting solution was refluxed for three hours under argon, and the solution was cooled to room temperature. To this
30 freshly prepared Grignard reagent solution was added Weinreb amide [Nahm et al., Tetrahedron Lett., 22:3815-3818 (1981); Angelastro et al., J. Org. Chem., 54:3913-3916 (1989)] (1.65 g, 4.0 mmol) portionwise under a positive argon pressure and stirred at room temperature
35 for three hours before pouring into a saturated NH₄Cl

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solution at zero degrees C. The product was extracted with ethyl acetate (2X) and purified on silica gel column (20 percent EA in H) to afford Compound 37 as a pale yellow oil (2.0 g, 92 percent): TLC (R_f 0.35, 25 percent EA in H, UV or AMA); NMR ($CDCl_3$, 400 MHz), δ 1.42 (s, 9H), 1.54-1.85 (m, 6H), 2.86 (dd, $J=5.6$, 14 Hz, 1H), 3.12 (dd, $J=5.6$, 14 Hz, 1H), 3.57 (m, 1H), 3.91 (m, 1H), 4.53 (m, 1H), 4.72 (m, 1H), 4.81 (m, 1H), 5.0 (s, 2H), 5.39 (d, $J=8$ Hz, 1H), 5.48 (m, 1H), 6.81 (d, $J=8.4$ Hz, 2H), 6.90 (d, $J=8.4$ Hz, 2H), 7.25-7.39 (m, 5H), 7.42 (t, $J=8$ Hz, 1H), 7.52 (d, $J=8$ Hz, 1H), 7.80 (d, $J=8$ Hz), 7.92 (s, 1H).

(B) Compound 38

Compound 37 (0.6 g, 1.1 mmol) in 10 mL MeOH was treated with 1 drop of 6N HCl and stirred at room temperature for two hours. The reaction was diluted with ethyl acetate and washed with 5 percent $NaHCO_3$ (2x) and dried over $MgSO_4$. The corresponding alcohol was obtained as a white wax (500 mg, 98 percent) after removal of the solvent: TLC (R_f 0.55, 50 percent EA in H, UV or AMA). The benzylic alcohol (210 mg, 0.46 mmol) was oxidized to aldehyde Compound 38 (195 mg, 93 percent) with pyridinium dichromate [PDC; Corey et al., Tetrahedron Lett., 5:399-402 (1979) (400 mg, 1.1 mmol) in dry DMF (4 mL) and purified by silica G column (35 percent EA in H): TLC (R_f 0.3, 25 percent EAZ in H, UV or AMA); NMR ($CDCl_3$, 400 MHz), δ 1.42 (s, 9H), 2.97 (dd, $J=5.6$, 14 Hz, 1H), 3.12 (dd, $J=6.4$, 13.6 Hz, 1H), 4.98 (s, 2H), 5.36 (d, $J=8$ Hz, 1H), 5.49 (m, 1H), 6.80 (d, $J=8.4$ Hz, 2H), 6.92 (d, $J=8.8$ Hz, 2H), 7.31-7.38 (m, 5H), 7.61 (t, $J=8$ Hz, 1H), 8.06 (d, $J=8$ Hz, 1H) 8.14 (d, $J=8$ Hz, 1H), 8.34 (s, 1H), 10.03 (s, 1H).

(C) Compound 28

To a heterogeneous solution of AgO (124 mg, 1 mmol) in 2 mL 1N NaOH was added aldehyde Compound 38 in

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4 mL MeOH. The reaction mixture was stirred at room temperature for 30 minutes. TLC showed disappearance of the starting material. The reaction mixture was diluted with ethyl acetate and the organic layer was washed with 1N HCl and dried over MgSO_4 . The Boc-protected Compound 28 was obtained as a pale yellow powder (160 mg, 86 percent) after removal of the solvent and lyophilization. The protected Compound 28 (160 mg, 0.34 mmol) was dissolved in 15 mL dry ether and deprotected according to the Method B (2) to give Compound 28 as a white powder (110 mg, 80 percent, HCl salt: NMR (DMSO, 500 MHz), δ 3.10 (m, 2H), 5.03 (s, 2H), 5.45 (br s, 1H), 6.86 (d, $J=8.7$ Hz, 2H), 7.03 (d, $J=8.6$ Hz, 2H), 7.34-7.43 (m, 5H), 7.64 (t, $J=7.7$ Hz, 1H), 8.21 (m, 2H), 8.38 (s, 1H), 8.48 (br m, 3H); HSMS: 376.1549 (M+H)⁺, calcd for ($\text{C}_{23}\text{H}_{21}\text{NO}_4+\text{H}$) 376.1549.

Example 20: Preparation of Compound 29

The title compound was prepared by first coupling Weinreb amide [Nahm et al., Tetrahedron Lett., 22:3815-3818 (1981); Angelastro et al., J. Org. Chem., 54:3913-3916 (1989)] with 4-bromobenzotrifluoride according to Method G and deprotected according to Method B (2) to give Compound 29 as a pale yellow solid (HCl salt): NMR (DMSO, 400 MHz), δ 3.13 (t, $J=6.8$ Hz, 2H), 5.02 (s, 2H), 5.44 (br s, 1H), 6.85 (d, $J=8.6$ Hz, 2H), 7.04 (d, $J=8.6$ Hz, 2H), 7.33-7.41 (m, 5H), 7.89 (d, $J=8.2$ Hz, 2H), 8.13 (d, $J=8.2$ Hz, 2H), 8.61 (br s, 3H); HRMS: 400.1540 (M+H)⁺, calcd for ($\text{C}_{23}\text{H}_{20}\text{F}_3\text{NO}_2+\text{H}^+$) 400.1524.

Example 21: Preparation of Compound 44 (Scheme 4)

(A) 3(S)-1,4-Diphenyl-2-oxo-3-amino-N-Boc-butane (Compound 40)

To a stirred solution of N-Boc-L-phenylalanine-N-methoxy-N-methylamide (Compound 39) (5.0 g, 14.5 mmol)

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in anhydrous THF (50 mL) under N₂ at zero degrees C was added 2.0 M benzyl magnesium chloride in THF (21.7 mL, 43.5 mmol). The mixture was gradually warmed to room temperature and stirred for an additional three hours. The reaction mixture was then poured onto 1 N HCl (25 mL). The organic layer was separated and the aqueous layer was extracted with ether (3 x 35 mL). The combined organic layers were dried (MgSO₄) and concentrated to give a crude product. Purification of the crude material by flash chromatography (EA:H;1:4) afforded Compound 40 as a white solid (4.8 g, 98 percent). R_f 0.3 (EA:H;1:4); mp 86-87°C; [α]_D²⁵ +31.22° (c 2.21, CH₂Cl₂); IR 3485, 2978, 1709, 1704, 1490, 1363, 1250 cm⁻¹; ¹H-NMR (CDCl₃) δ 1.14 (s, 9H), 2.9-3.15 (m, 2H), 3.65 (q, 2H, J=11.6 Hz), 4.61 (d, 1H, J=6.9 Hz), 5.1 (bs, 1H), 7.0-7.2 (m, 10H) ppm; ¹³C (CDCl₃) δ 28.3, 37.8, 47.8, 59.5, 79.9, 127.0, 127.1, 128.8, 129.2, 129.6, 133.1, 135.2, 155.1, 206.5 ppm. HRMS: 472.0880, calcd for C₂₁H₂₅NO₃+Cs⁺: 472.0889.

(B) (2R,3S)-1,4-Diphenyl-2-hydroxy-3-amino-N-Boc-butane (Compound 41)

To a stirred solution of Compound 40 (0.49 g, 1.4 mmol) in anhydrous MeOH (1 mL) under N₂ at -22°C was added NaBH₄ (0.16 g, 4.4 mmol). After 30 minutes, the mixture was gradually warmed to room temperature and then poured onto saturated NH₄Cl (10 mL). The mixture was extracted with ether (5 x 25 mL). The combined organic phase was washed with 1 N HCl (2 x 25 mL), saturated NaHCO₃ (3 x 25 mL), dried (MgSO₄) and concentrated to give a crude product as a 9:1 mixture of diastereomers as determined by ¹H-NMR.

Recrystallization from benzene afforded Compound 41 as a crystalline white solid (0.42 g, 80 percent). R_f 0.26 (EA:H;1:4); mp 157-158°C; [α]_D²⁵ -10.12° (c 0.5, CH₂Cl₂); IR 3691, 3155, 2927, 1794, 1706, 1471, 1381, 1166, 1096

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cm⁻¹; ¹H-NMR (CDCl₃, 300 MHz), δ 1.35 (s, 9H), 2.55-3.05 (m, 4H), 3.83-4.0 (m, 2H), 4.65 (bs, 1H), 7.05-7.2 (m, 10H) ppm; ¹³C (CDCl₃, 125 MHz) δ 28.3, 35.4, 40.3, 56.1, 74.8, 77.3, 126.4, 126.6, 128.4, 128.7, 129.3, 129.4, 138.1, 193.2 ppm. HRMS: 364.1902, calcd for C₂₃H₂₅NO₃+H⁺: 364.1913.

The product was derivatized by treatment with SOCl₂ to give the 3,4-dibenzyl-oxazolidinone. [Kano et al., *Tetrahedron Lett.*, 28:6331-6334 (1987).] The assignment of stereochemistry was based on the vicinal coupling, J_{3,4}=5.0 Hz, consistent with that of the trans oxazolidinone.

(C) (2R,3S)-1,4-Diphenyl-2-hydroxy-3-amino-butane HCl (Compound 44)

To a stirred solution of Compound 41 (0.20 g, 0.58 mmol) in CH₂Cl₂ (5 mL) was added TFA (0.23 mL, 2.9 mmol). After 12 hours, the mixture was concentrated to give a crude white solid, which was taken up in 1N HCl (1 mL) and concentrated to give a crude product. This procedure was repeated three times. Recrystallization from ether/MeOH afford Compound 31 as a white crystalline solid (0.14 g, 87 percent): mp 158-159°C; [α]_D²⁵ -11.83° (c 3.21, MeOH); ¹H-NMR (CD₃OD, 300 MHz) δ 2.6-2.8 (m, 3H), 3.05 (dd, 2H, J=10.0, 15.3 Hz), 3.2-3.3 (m, 1H), 3.43 (q, 2H, J=7.0 Hz), 3.88 (sept, 1H, J=3 Hz), 7.0-7.2 (m, 10H) ppm; ¹³C-NMR (CD₃OD, 125 MHz) δ 33.9, 40.0, 58.1, 72.9, 127.7, 128.5, 129.7, 130.2, 130.3, 130.4, 137.4, 139.4 ppm. HRMS: 264.6601, calcd for C₁₆H₂₀NO⁺+Na⁺: 264.6611.

Example 22: Preparation of Compound 45 (Scheme 4)

(A) (7S)-6-Oxo-7-amino-8-phenyl-N-Boc-octene (Compound 42)

To a stirred solution of Compound 39 (2.3 g, 7.4 mmol) in anhydrous ether (30 mL) under N₂ at zero

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degrees C was added freshly prepared pentene magnesium bromide (6.5 g, 37.3 mmol) in anhydrous ether (20 mL). The mixture was gradually warmed to room temperature and stirring was continued for 18 hours. The reaction mixture was poured onto 1 N HCl (30 mL) at zero degrees C. The organic layer was separated and the aqueous layer was extracted with ether (4 x 25 mL). The combined organic phase was washed successively with 1 N HCl x 25 mL, saturated NaHCO₃ (2 x 25 mL), saturated NaCl (1 x 25 mL), dried over (MgSO₄), and concentrated to give a crude white solid. Purification by flash chromatography (EA:H;1:2) gave Compound 42 as a white crystalline solid (2.4 g, 98 percent). R_f 0.7 (EA:H;1:1); mp 77.5-79°C; [α]_D²⁵ +50.58° (c 3.16 CH₂Cl₂); IR 3436, 2978, 2932, 1708, 1705, 1490, 1367, 1249, 1171 cm⁻¹; ¹H-NMR (CDCl₃, 300 MHz) δ 1.41 (s, 9H), 1.64 (s, 2H, J=6.8 Hz), 2.0 (q, 2H, J=7.1 Hz), 2.27-2.47 (m, 2H), 3.0 (dq, 2H, J=6.7, 13.9 Hz), 4.52 (q, 1H, J=7.1 Hz), 4.94-5.09 (m, 2H), 5.1 (bd, 1H, J=7.1 Hz), 5.64-5.77 (m, 1H), 7.0-7.2 (m, 5H) ppm; ¹³C-NMR (CDCl₃, 125 MHz) δ 22.2, 28.2, 32.8, 37.8, 39.8, 59.9, 79.7, 115.2, 126.9, 128.5, 129.1, 136.2, 137.7, 155.1, 209.1 ppm. HRMS: 450.1030, calcd for C₁₉H₂₇NO₃+Cs⁺: 450.1045.

(B) (6S)-5-Oxo-6-amino-7-phenyl-N-Boc-heptanoic acid (Compound 43)

To a stirred solution of Compound 42 (0.23 g, 0.73 mmol) in acetone (20 mL) and water (20 mL) at room temperature was added NaIO₄ (0.62 g, 2.93 mmol) and KMnO₄ (0.07 g, 0.46 mmol). After an additional 12 hours of stirring the mixture was filtered and the resulting filtrate was concentrated. The concentrate was treated with 0.01 N NaOH (20 mL) and washed with ether (2 x 10 mL). The aqueous layer was then treated with 1 N HCl until acidic. The aqueous layer was then extracted with ether (4 x 15 mL). These organic layers were combined,

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dried over (MgSO₄) and then concentrated to give a crude oil. Purification by chromatography (EA:H:1:4; 0.1 percent AcOH) afforded Compound 43 as a white solid (0.19 g, 78 percent). R_f 0.2 (EA:H:0.1 percent AcOH); mp 88-89°C; [α]_D²⁵ +8.32° (c 2.88, CH₂Cl₂); IR 3691, 3553, 2983, 1794, 1708, 1472, 1381, 1166, 1097 cm⁻¹; ¹H-NMR (CDCl₃, 300 MHz) δ 1.37 (s, 9H), 1.75-1.90 (m, 2H), 2.2-2.6 (m, 4H), 2.9-3.1 (m, 2H), 4.55 (q, 1H), 5.1 (bd, 1H), 7.0-7.3 (m, 5H) ppm; ¹³C-NMR (CDCl₃, 125 MHz) δ 18.1, 28.3, 32.7, 37.9, 39.5, 60.0, 80.1, 27.1, 128.7, 129.2, 136.1, 155.3, 178.2, 208.7 ppm. HRMS: 358.1630, calcd for C₁₈H₂₅NO₃+Na⁺: 358.1630.

(C) (6S)-5-Oxo-6-amino-7-phenyl-heptanoic acid HCl (Compound 45)

To a stirred solution of Compound 43 (0.17 g, 0.5 mmol) in CH₂Cl₂ (3 mL) was added TFA (0.4 mL, 5.1 mmol). After 12 hours, the mixture was concentrated to give a crude white solid, which was taken up in 1 N HCl (1 mL) and concentrated. This was repeated three times. The crude product was recrystallized from MeOH/EA to give Compound 45 as a white crystalline solid (0.12 g, 88 percent). mp 154-155°C; [α]_D²⁵ +10.68° (c 1.03, MeOH); ¹H-NMR (D₆-DMSO, 300 MHz) δ 1.5-1.8 (m, 2H), 2.15 (dt, 2H, J=2.2, 7.0 Hz), 2.4-2.6 (m, 2H), 3.1 (d, 2H, J=7.0 Hz), 3.34 (s, 4H), 4.36 (t, 1H, J=7.0 Hz), 7.25-7.30 (m, 5H) ppm; ¹³C-NMR (DMSO, 125 MHz) δ 18.0, 32.4, 35.3, 38.94, 58.5, 127.3, 128.7, 129.4, 134.8, 174.0, 206.0 ppm. HRMS: 236.2801, calcd for C₁₃H₁₈NO₃Cl⁺: 236.2870.

Example 23: Preparation of Compound 27 (Scheme 5)

(A) (2S)-3-[4-(Benzyloxy)phenyl]-2-amino-N-Boc-propanol (Compound 48)

To a stirred solution of N-Boc-L-tyrosine (Compound 46; 4.0 gr, 14.2 mmol) in DMF (20 mL) at room temperature was added benzyl bromide (3.7 mL, 30.6

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mmol), Cs_2CO_3 (13.9 gr, 42.6 mmol) and TBAI (25 mg, 0.07 mmol). Stirring was continued for 24 hours after which the reaction was taken up in EA (100 mL). The organic layer was washed with 1 N HCl (3 x 20 mL), saturated NaHCO_3 (3 x 20 mL) and saturated NaCl (1 x 20 mL). The organic layer was dried (MgSO_4) and concentrated to give a crude yellow solid. Purification by flash chromatography (EA:H;1:4) afforded O-benzyl-L-tyrosine-N-Boc-benzyl ester (Compound 47) as a white solid (3.0 gr, 46 percent). ^1H NMR (500 MHz, CDCl_3) δ 1.42 (s, 9 H), 3.0 (d, 2 H, $J = 3.1$ Hz), 4.58 (q, 1 H, $J = 5.8$ Hz), 4.97 (q, 1 H, $J = 8.3$ Hz), 5.0 (s, 2 H), 5.15 (q, 2 H, $J = 12.3$ Hz), 6.9 (AB, 4 H, $J = 8.4$, 53.5 Hz), 7.29-7.43 (m, 5 H) ppm; ^{13}C NMR (125 MHz, CDCl_3) δ 28.3, 37.3, 54.5, 67.0, 69.9, 79.9, 114.8, 127.4, 127.9, 128.4, 128.5, 130.3, 135.0, 136.9, 155.1, 157.8, 171.7 ppm.

To a stirred solution of Compound 47 (2.7 gr, 5.9 mmol) in anhydrous THF (15 mL) at room temperature under N_2 was added LiBH_4 (0.07 gr, 3.2 mmol). After two hours the reaction mixture was poured onto 1 N HCl (5 mL) and then taken up in EA (50 mL). The organic layer was washed with 1 N HCl (3 x 5 mL), saturated NaHCO_3 (3 x 5 mL) and saturated NaCl (1 x 5 mL). The organic layer was dried (MgSO_4) and concentrated to give a crude white solid. Purification by flash chromatography (EA:H;1:4) afforded the Compound 48 as a white solid (2.0 gr, 97 percent). ^1H NMR (500 MHz, CDCl_3) δ 1.42 (s, 9 H), 2.78 (d, 2 H, $J = 6.8$ Hz), 2.84 (br s, 1 H), 3.53-3.62 (m, 1 H), 3.63-3.65 (m, 1 H), 3.82 (bs, 1 H), 4.86 (br s, 1 H), 5.03 (s, 2 H), 6.9 (AB, 4 H, $J = 8.5$, 53.9 Hz), 7.29-7.43 (m, 5 H) ppm; ^{13}C NMR (125 MHz, CDCl_3) δ 28.3, 36.3, 54.5, 64.8, 79.6, 114.8, 127.4, 127.9, 128.5, 128.5, 130.3, 136.9, 157.8 ppm.

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(B) (2S)-O-Tosyl-3-[4-(benzyloxy)phenyl]-2-amino-N-Boc-propanol (Compound 49)

To a stirred solution of Compound 48 (2.0 gr, 5.6 mmol) in anhydrous CH_2Cl_2 (10 mL) at zero degrees C under N_2 was added Et_3N (1.2 mL, 8.4 mmol), DMAP (cat.) and tosyl chloride (1.4 gr, 7.3 mmol). After 5 hours, the reaction mixture was washed with 1 N HCl (2 x 5 mL), saturated NaHCO_3 (2 x 5 mL) and saturated NaCl (1 x 5 mL). The organic layer was dried (MgSO_4) and concentrated to give a crude yellow solid. Purification by flash chromatography gave the Compound 49 as a white solid (2.2 gr, 79 percent). ^1H NMR (300 MHz, CDCl_3) δ 1.40 (s, 9 H), 2.45 (s, 3 H), 2.60-2.81 (m, 2 H), 3.75-4.0 (m, 3 H), 4.7 (bd, 1 H, $J = 7.5$ Hz), 5.0 (s, 2 H), 6.92 (AB, 4 H, $J = 8.4, 45.4$ Hz), 7.34 (dd, 6 H, $J = 6.5, 8.2, 15.9$ Hz), 7.78 (d, 2 H, $J = 8.5$ Hz) ppm.

(C) (2S)-S-Acetate-3-[4-benzyloxy)phenyl]-2-amino-N-Boc-propanethiol (Compound 50)

To a stirred solution of Compound 49 (2.1 gr, 4.2 mmol) in anhydrous DMF (10 mL) at zero degrees C under N_2 was added potassium thioacetate (0.6 gr, 5.04 mmol). After three hours, the reaction was gradually allowed to warm to room temperature. After an additional 8 hours of stirring at room temperature, the reaction mixture was taken up in EA (50 mL). The organic layer was washed with 1 N HCl (3 x 20 mL), saturated NaHCO_3 (3 x 20 mL) and saturated NaCl (1 x 20 mL). The organic layer was dried (MgSO_4) and concentrated to afford a crude brownish solid. Recrystallization from EA/H gave the Compound 50 as white solid (1.6 gr, 93 percent). ^1H NMR (CDCl_3 , 500 MHz) δ 1.39 (s, 9 H), 2.35 (s, 3 H), 2.70 (dd, 1 H, $J = 3.6, 6.6$ Hz), 2.73-2.89 (m, 2 H), 2.91 (dd, 1 H, $J = 4.0, 7.0$ Hz), 3.93 (bs, 1 H), 4.56 (bs, 1 H), 5.03 (s, 2 H), 7.02 (AB, 4 H, $J = 4.4, 38.0$ Hz), 7.31-7.51 (m, 5 H)

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ppm. HRMS: 548.0888, Calcd for $C_{23}H_{27}NSO_4 + Cs^+$: 548.0872.

(D) (2S)-3-[4-(benzyloxy)phenyl]-2-amino-
propanethiol HCl (Compound 27)

5 To a stirred solution of Compound 50 (0.1 gr, 0.24 mmol) in ethanol (5 mL) at zero degrees C was added 1 N NaOH solution (0.48 mL, 0.48 mmol). After one hour the reaction mixture was gradually warmed to room temperature and stirred for an additional 3 hours. The
10 reaction mixture was then taken up in EA (50 mL). The organic solution was washed 1 N HCl (3 x 25 mL), saturated $NaHCO_3$ (3 x 25 mL) and saturated NaCl (1 x 25 mL). The organic layer was dried ($MgSO_4$) and concentrated to give a crude white solid of Compound 51
15 (0.09 gr, > 99 percent). R_f 0.31 (EA:H;1:4); 1H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 9 H), 2.5-2.9 (m, 4 H), 3.95 (bs, 1 H), 4.72 (bs, 1 H), 5.03 (s, 2 H), 6.9-7.1 (AB, 4 H), 7.3-7.5 (m, 5 H) ppm.

The crude white solid Compound 51 (0.09 gr, 2.8
20 mmol) was taken up in ether (1 mL). To the stirred solution was added a saturated HCl (g)/ether solution (10 mL). After 24 hours of stirring at room temperature, the solvent was removed to give another crude white solid. Recrystallization from MeOH/ether
25 afforded the Compound 27 as a white solid (0.03 gr, 34 percent). 1H NMR (300 MHz, $DMSO-d_6$) δ 2.62 (dd, 1 H, $J = 6.2, 14.7$ Hz), 2.82 (dd, 1 H, $J = 4.6, 14.7$ Hz), 2.84-2.95 (m, 2 H), 3.52 (p, 1 H, $J = 7.0$ Hz), 5.07 (s, 2 H), 6.97 (AB, 4 H, $J = 8.6, 19.8$ Hz), 7.3-7.45 (m, 5 H) ppm.
30 HRMS: 274.1258, Calcd for $C_{16}H_{20}NSO (M^+)$: 274.1266. Anal Calcd for $C_{16}H_{20}NSOCl$: C 62.02 percent, H 6.51 percent, N 4.52 percent, S 10.34 percent. Found C 62.11 percent, H 6.59 percent, N 4.31 percent, S 10.00 percent.

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(E) Preparation of Compound 27a (Scheme 5)(1) Di-((2S)-3-[4-(benzyloxy)phenyl]-2-amino-N-Boc-propane)-disulphide (Compound 51a)

5

To a stirred solution of Compound 50 (0.3 g, 0.72 mmol) in ethanol (5 mL) at zero degrees C was added 1 N NaOH solution (1.44 mL, 1.42 mmol). After one hour, the reaction mixture was gradually warmed to room temperature, and stirred for an additional 3 hours. Then an I₂/ethanol solution was added until a yellowish solution persisted, and stirring was continued for 30 minutes. The reaction mixture was then taken up in EA (100 mL). The organic solution was washed with a 20 percent Na₂S₂O₃ solution until the organic layer was clear, followed by 1 N HCl (3 x 25 mL), saturated NaHCO₃ (3 x 25 mL) and saturated NaCl (1 x 25 mL). The organic layer was dried (MgSO₄) and concentrated to give a crude white solid. Recrystallization from EA/ether afforded Compound 51a as a white solid (0.22 g, 97 percent). ¹H NMR (CDCl₃, 300 MHz) δ 1.41 (s, 18 H), 2.7-3.0 (m, 8 H), 4.0-4.1 (bs, 2 H), 5.03 (s, 2 H), 7.0 (AB, 8 H, J = 8.5, 58.1 Hz), 7.25-7.3 (m, 10 H) ppm.

20

(2) Di-((2S)-3-[4-(benzyloxy)phenyl]-2-amino-propane)-disulphide HCl (Compound 27a)

To a stirred solution of Compound 51a (0.2 g, 1.6 mmol) in ether (5 mL) was added a saturated HCl (g)/ether solution (10 mL). After 24 hours, the resulting precipitate was isolated by filtration and recrystallization from MeOH/ether to afford Compound 27a as a white solid (0.06 g, 30 percent). ¹H NMR (500 MHz, CD₃OD) δ 2.78-2.88 (m, 4 H), 2.9 (dd, 2 H, J = 4.2, 7.0 Hz), 3.01 (dd, 2 H, J = 3.0, 7.0), 3.75-3.77 (bs, 2 H), 5.1 (s, 4 H), 7.1 (AB, 8 H, J = 4.3, 34.0 Hz), 7.34-7.45 (m, 5 H) ppm. HRMS: 545.2311, Calcd for C₂₃H₃₈N₂S₂O₂Cl₂ + H⁺: 545.2296.

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Example 24: Preparation of Compound 54(A) (2S)-1-[4-(Benzyloxy)phenyl]-6-phenyl-2-amino-3-oxo-N-Boc-hexane-(Compound 53)

To a stirred solution of N-Boc-O-benzyl-L-tyrosine-N-methoxy-N-methylamide (Compound 52; 1.5 gr, 3.62 mmol) in anhydrous THF (10 mL) under N₂ at room temperature was added freshly prepared 3-phenyl-propane magnesium bromide (10.9 mmol) in anhydrous THF (10 mL). Stirring was continued for 3 hours after which time the reaction was poured onto 1 N HCl (30 mL). The organic layer was separated and the aqueous layer was extracted with EA (4 x 25 mL). The combined organics were washed with 1 N HCl (3 x 25 mL), saturated NaHCO₃ (3 x 25 mL) and saturated NaCl (1 x 25 mL). The organic layer was dried (MgSO₄) and concentrated to give a crude yellowish solid. Recrystallization from EA:H (1:15) gave Compound 53 as a white crystalline solid (1.35 gr, 80 percent). mp 128-129°C; ¹H NMR (CDCl₃, 500 MHz) δ 1.40 (s, 9 H), 1.78-1.95 (m, 2 H), 2.25-2.48 (m, 2 H), 2.56 (t, 2 H, J = 14.0 Hz), 2.96 (dd, 1 H, J = 6.8, 14.0 Hz), 4.46 (q, 1 H, J = 7.5 Hz), 5.05 (s, 2 H), 5.10 (d, 1 H, J = 7.5 Hz), 6.88-7.13 (AB, 4 H), 7.2-7.4 (m, 10 H) ppm; ¹³C (CDCl₃, 125 MHz) δ 24.7, 28.3, 34.9, 37.0, 40.0, 60.1, 70.0, 79.0, 114.9, 125.9, 127.4, 128.4, 128.5, 128.6, 130.2, 136.9, 141.3, 155.15, 157.77, 209.16 ppm. HRMS: 472.2490, Calcd for C₃₀H₃₃NO₄ + H⁺: 472.2488.

(B) (2S)-1-[4-(Benzyloxy)phenyl]-6-phenyl-2-amino-3-oxo-hexane HCl (Compound 54)

To a stirred solution of Compound 53 (0.1 gr, 0.21 mmol) in ether (5 mL) at room temperature was added a saturated HCl (g)/ether solution (20 mL). After 24 hours of stirring, the resulting precipitate was isolated by filtration. The crude product was recrystallized from MeOH/ether to give the Compound 54 as hard white crystals (0.065 gr, 75 percent). ¹H NMR

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(300 MHz, DMSO- d_6) δ 1.65-1.78 (m, 2 H), 2.49-2.55 (m, 4 H), 3.0 (d, 2 H, J = 6.5 Hz), 4.31 (t, 1 H, J = 6.5 Hz), 5.07 (s, 2 H), 6.92-7.8 (m, 14 H), 8.2 (br s, 3 H) ppm; ^{13}C NMR (125 MHz, DMSO- D_6) δ 24.4, 34.6, 34.8, 58.7, 68.2, 115.0, 125.9, 126.8, 127.7, 127.8, 128.3, 128.4, 128.5, 130.6, 137.1, 157.8, 208.3 ppm. HRMS: 374.2106, Calcd for $\text{C}_{25}\text{H}_{28}\text{NO}_2\text{Cl} + \text{H}^+$: 374.2120. Anal Calcd for $\text{C}_{25}\text{H}_{28}\text{NO}_2\text{Cl}$: C 73.25 percent, H 6.88 percent, N 3.42 percent. Found C 73.25 percent, H 6.81 percent, N 3.40 percent.

Example 25: Preparation of Compound 60 (Scheme 6)

(A) O-(2-Naphthylmethyl)-L-tyrosine-N-Boc-(2-naphthylmethyl) ester (Compound 55)

To a stirred solution of N-Boc-L-tyrosine (Compound 46) 6.1 gr, 21.4 mmol) in DMF (30 mL) at room temperature was added 2-(bromomethyl)naphthalene (10.4 gr, 47.0 mmol), Cs_2CO_3 (17.4 gr, 53.4 mmol) and TBAI (25 mg, 0.07 mmol). Stirring was continued for 48 hours after which the reaction was taken up in EA (200 mL). The organic layer was washed with 1 N HCl (3 x 240 mL), saturated NaHCO_3 (3 x 40 mL) and saturated NaCl (1 x 40 mL). The organic layer was dried (MgSO_4) and concentrated to give a crude brown oil.

Recrystallization from MeOH afforded Compound 55 as a white solid (5.0 gr, 42 percent). m.p. 99.5-101°C; ^1H NMR (CDCl_3 , 300 MHz) δ 1.41 (s, 9 H), 3.02 (d, 2 H, J = 5.7 Hz), 4.62 (q, 1 H, J = Hz), 5.01 (br d, 1 H, J = Hz), 5.07 (s, 2 H), 5.23 (d, 1 H, J = 12.2 Hz), 5.34 (d, 1 H, J = 12.3 Hz), 6.83 (AB, 4 H, J = 8.4, 43.2 Hz), 7.4-7.53 (m, 6 H), 7.8-7.99 (m, 8 H) ppm; ^{13}C NMR (CDCl_3 , 125 MHz) δ 28.3, 38.3, 55.0, 67.2, 70.2, 78.2, 125.3, 126.1, 126.2, 126.3, 126.4, 126.5, 127.7, 127.8, 127.9, 128.1, 128.4, 130.4, 132.6, 133.07, 133.13, 133.19,

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133.29, 134.48, 157.85, 171.57 ppm. HRMS: 694.1570,
Calcd for $C_{36}H_{35}NO_5 + Cs^+$: 694.1570.

(B) (2S)-3-[4-(2-Naphthylmethyloxy)phenyl]-2-amino-N-Boc-propanol (Compound 56)

5 To a stirred solution of Compound 55 (1.5 gr,
2.6 mmol) in anhydrous THF (25 mL) at room temperature
under N_2 was added $LiBH_4$ (0.058 gr, 2.67 mmol). After 18
hoursn the reaction mixture was poured onto 1 N HCl (10
10 mL). The solvent was reduced and the resulting slurry
was taken up in EA (50 mL). The organic layer was
washed with 1 N HCl (3 x 25 mL), saturated $NaHCO_3$ (3 x
25 mL) and saturated NaCl (1 x 25 mL). The organic
layer was dried ($MgSO_4$) and concentrated to give a crude
white solid. Purification by flash chromatography
15 (EA:H;1:4) afforded Compound 56 as a white solid (1.0
gr, 96 percent). R_f 0.44 (EA:H;1:1): m.p. 138-139°C;
[α] $^{25}_D$ -13.89° (c 1.8, CH_2Cl_2); 1H NMR ($CDCl_3$, 300 MHz) δ
1.40 (s, 9 H), 2.76 (d, 2 H, $J = 7.2$ Hz), 3.51 (dd, 1 H,
 $J = 5.1, 10.9$ Hz), 3.63 (dd, 1 H, $J = 3.6, 11.2$ Hz), 3.8
20 (bs, 1 H), 4.78 (bd, 1 H, $J = 7.8$ Hz), 5.18 (s, 2 H),
7.0 (AB, 4 H, $J = 8.4, 53.1$ Hz), 7.4-7.6 (m, 3 H), 7.7-
7.9 (m, 4 H) ppm. HRMS:540.1153, Calcd for $C_{25}H_{29}NO_4 + Cs^+$: 540.1151. Anal Calcd for $C_{25}H_{29}NO_4$: C 73.68 percent,
H 7.17 percent, N 3.43 percent. Found C 73.60 percent,
25 H 7.21 percent, N 3.61 percent.

(C) (2S)-O-Mesyl-3-[4-(2-naphthylmethyloxy)phenyl]-2-amino-N-Boc-propanol (Compound 57)

To a stirred solution of Compound 56 (1.0 gr,
30 2.45 mmol) in anhydrous CH_2Cl_2 (20 ml) at zero degrees C
under N_2 was added Et_3N (0.52 mL, 3.7 mmol), DMAP (cat.)
and mesyl chloride (0.29 mL, 3.7 mmol). After one hour,
the reaction was quenched by addition of H_2O (5 mL).
The organic layer was separated and the aqueous layer
35 was extracted with CH_2Cl_2 (3 x 10 mL). The combined
organics were washed with 1 N HCl (2 x 20 mL), saturated

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NaHCO₃ (2 x 20 mL) and saturated NaCl (1 x 20 mL). The organic layer was dried (MgSO₄) and concentrated to give a white solid. Purification by flash chromatography (EA:H;1:2) afforded Compound 57 as a white solid (0.92 gr, 78 percent). m.p. 112.5-113.5°C; [α]_D²⁵ -12.03 (c 1.33, CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 1.41 (s, 9 H), 2.78-2.82 (m, 2 H), 2.99 (s, 3 H), 4.0-4.25 (m, 3 H), 4.6-4.65 (bd, 1 H), 5.2 (s, 2 H), 6.9-7.2 (m, 4 H), 7.45-7.08 (m, 3 H), 7.8-7.9 (m, 4 H) ppm; ¹³C (125 MHz, CDCl₃) δ 28.3, 37.2, 36.25, 50.9, 69.7, 70.2, 115.2, 125.2, 126.3, 126.4, 127.7, 127.9, 128.4, 128.9, 130.3, 133.1, 133.3, 134.4, 157.8 ppm. HRMS:485.1870, Calcd for C₂₆H₃₁NSO₆ (M⁺): 485.1872.

(D) (2S)-3-[4-(2-Naphthylmethoxy)phenyl-2-amino-N-Boc-azido-propane (Compound 58)]

To a stirred solution of mesylate (Compound 57) (0.9 gr, 1.85 mmol) in DMF (10 mL) at 60°C was added NaN₃ (0.72 gr, 11.1 mmol). After 3 hours the reaction mixture was taken up in EA (50 mL) and then washed with 1 N HCl (2 x 20 mL), saturated NaHCO₃ (2 x 20 mL) and saturated NaCl (1 x 20 mL). The organic layer was dried (MgSO₄) and concentrated. The crude white solid was purified by flash chromatography to give Compound 58 as a white solid (0.6 gr, 76 percent). m.p. 141-142°C; [α]_D²⁵ -5.8° (c 1.72, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 1.44 (s, 9 H), 2.7-2.78 (dd 1 H, J = 8.0, 14.0 Hz), 2.8-2.9 (d, 1 H, J = 6.0 Hz), 3.28-3.33 (dd, 1 H, J = 4.0, 12.0 Hz), 3.41-3.49 (m, 1 H), 3.94 (s, 1 H), 4.68 (bd, 1 H, J = 7.0 Hz), 5.22 (s, 3 H), 6.95-7.2 (AB, 4 H, J = 8.0, 75.0 Hz), 7.48-7.76 (m, 3 H), 7.8-7.9 (m, 4 H) ppm; ¹³C (125 MHz, CDCl₃) δ 28.3, 37.2, 51.4, 53.0, 79.68, 115.0, 125.2, 126.0, 126.2, 126.3, 127.7, 127.9, 128.3, 129.4, 130.3, 133.0, 133.2, 134.4, 155.0, 157.6 ppm; IR (KBr) 3373, 2981, 2943, 2861, 2361, 2103, 1683, 1611,

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1510, 1288, 1242, 1167 cm^{-1} . HRMS: 429.2139, Calcd for $\text{C}_{25}\text{H}_{30}\text{N}_2\text{O}_3 + \text{Na}^+$: 429.2154.

(E) (2S)-3-[4-(2-Naphthylmethyloxy)phenyl]-
1,2-diamino-N-Boc-propane (Compound 59)

5 To a stirred solution of Compound 58 (0.11 gr, 0.27 mmol) in anhydrous THF (5 mL) at room temperature under N_2 was added LiAlH_4 (0.010 gr, 0.26 mmol). After 30 minutes, 1 N HCl (1 mL) was added to the reaction mixture. The reaction mixture was concentrated and then
10 taken up in 1 N NaOH (3 mL). The aqueous layer was extracted with CH_2Cl_2 (4 x 10 mL). The combined organics were washed with saturated NaCl (2 x 10 mL), dried (MgSO_4) and concentrated to give a white solid. Purification by flash chromatography (CH_2Cl_2 : Et_3N ;10:0.5)
15 afforded Compound 59 as a white solid (0.07 gr, 63 percent). ^1H NMR (300 MHz, CDCl_3) δ 1.41 (s, 9 H), 2.58 (dd, 2 H, $J = 6.8, 13.1$ Hz), 2.72 (dt, 2 H, $J = 8.6, 13.2$ Hz), 3.75 (bs, 1 H), 4.73 (bd, 1 H, $J = 8.6$ Hz), 5.19 (s, 2 H), 6.9-7.2 (AB, 4 H), 7.4-7.6 (m, 3 H), 7.8-
20 7.9 (m, 4 H) ppm; ^{13}C NMR (500 MHz, CDCl_3) δ 28.3, 37.9, 44.5, 54.0, 70.1, 114.8, 125.2, 126.0, 126.2, 126.3, 127.7, 127.9, 128.3, 130.2, 133.0, 133.2, 134.5, 155.8, 157.4 ppm. HRMS: 429.2139, Calcd for $\text{C}_{25}\text{H}_{30}\text{N}_2\text{O}_3 + \text{Na}^+$: 429.2154.

25 (F) (2S)-3-[4-(2-Naphthylmethyloxy)phenyl]-
1,2-diamino-propane HCl (Compound 60)

To Compound 59 (0.06 gr, 0.15 mmol) suspended in ether (1 mL) was add a saturated solution of HCl (g)/ether (5 mL). After 24 hours the reaction mixture
30 was concentrated and the crude solid recrystallized from MeOH/ether to give the desired product Compound 60 as a white solid (0.02 gr, 34 percent). ^1H NMR (500 MHz, CD_3OD) δ 2.92 (dd, 1 H, $J = 8.1, 14.7$ Hz), 3.03 (dd, 1 H, $J = 7.0, 14.8$ Hz), 3.19 (dd, 1 H, $J = 5.6, 14.0$ Hz),
35 3.27 (dd, 1 H, $J = 7.1, 13.9$ Hz), 3.75 (p, 1 H, $J = 7.0$

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Hz), 5.27 (s, 2 H), 7.12 (AB, 4 H, $J = 9.0$, 103.3 Hz), 7.42-7.61 (m, 3 H), 7.82-7.90 (m, 4 H) ppm.

HRMS: 307.1810, Calcd for $C_{20}H_{24}N_2O + H^+$: 307.1810.

5 Enzymatic Assays and Inhibition Analysis Based on the
Amidase Activity

Procedures and conditions for the enzymatic assays, kinetic analyses, and inhibition studies are essentially the same as previously reported and
10 indicated in Table 2.

Inhibition of the epoxide hydrolase activity of LTA₄
hydrolase

Aliquots of enzyme (2.5 mg) were preincubated
15 for 30-40 minutes at room temperature in 100 mL of 50 mM
HEPES, pH 8, containing various amounts of the
respective inhibitor. Each compound was added to the
buffer in DMF (final concentration < 0.25 percent) and in
the case of Compound 27, 1 mM dithiothreitol (DTT) was
20 also included in the incubation buffer. Samples were
further incubated with 20-25 mM LTA₄ for 15 seconds at
room temperature and the reactions were terminated by
the addition of 2 volumes of MeOH. After addition of
200 ng PGB₁ (internal standard), samples were acidified
25 to pH value of 3 with 0.1 M HCl, extracted on Chromabond
C18 columns (Mackerey & Nagel), and finally analyzed on
a computerized Waters HPLC system. The column (Radial-
Pak C₁₈ cartridge, 100 x 5 mm, Waters) was eluted with a
mixture of methanol/water/acetic acid (70:30:0.01, v/v)
30 at a flow rate of 1.2 mL/minutes. The UV absorbance of
the eluate was monitored continuously at 270 nm.
Amounts of LTB₄ were computer calculated from peak area
ratios between LTB₄ and the internal standard PGB₁.
Peak area ratios were converted into molar ratios from

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comparisons with standard curve constructed from injections of known amounts of the respective compound.

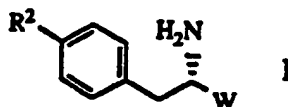
5 The foregoing description and the examples are intended as illustrative and are not to be taken as limiting. Still other variations within the spirit and scope of this invention are possible and will readily present themselves to those skilled in the art.

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Claims

1. A compound whose structure corresponds to the formula

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wherein the depicted -NH_2 group is in the (S) configuration; -W is $\text{-CH}_2\text{SH}$, $\text{-CH}_2\text{NH}_2$ or C(=Z)-Y , wherein =Z is =O , or -H and -OH ; and -Y is selected from the group consisting of (a) phenyl, (b) trifluoromethylphenyl, (c) carboxyphenyl, (d) benzyl, (e) $\text{C}_1\text{-C}_6$ alkylencarboxyl, (f) $\text{C}_1\text{-C}_6$ alkyl, (g) $\text{C}_2\text{-C}_6$ alkenyl, (h) $\text{C}_1\text{-C}_6$ alkylenephenyl and (i) -C(=O)-X-R^1 wherein X is O or NH and, R^1 is selected from the group consisting of $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_1\text{-C}_6$ alkylencarboxyl, and benzyl, with the proviso that -Y is other than (i) when Z is -H and -OH ; and;

R^2 is hydrogen, benzyloxy or 2-naphthylmethyloxy, and a pharmaceutically acceptable acid addition salt thereof.

2. The compound according to claim 1 wherein R^2 is benzyloxy.

3. The compound according to claim 2 that contains two phenyl rings.

4. The compound according to claim 1 wherein -W is $\text{-CH}_2\text{SH}$ or $\text{-CH}_2\text{NH}_2$.

5. The compound according to claim 1 wherein W is C(=Z)-Y .

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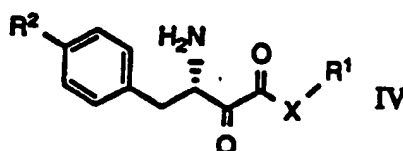
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6. The compound according to claim 5 wherein
=Z is =O.

7. The compound according to claim 6 wherein
-Y is -C(=O)-X-R¹.

8. A compound whose structure corresponds to
the formula

10



15 wherein the depicted -NH₂ group is in the
(S) configuration;

X is O or NH;

R¹ is selected from the group consisting
of C₁-C₆ alkyl, C₁-C₆ alkylencarboxyl and benzyl;

20 R² is hydrogen, benzyloxy or
2-naphthylmethyloxy; and

a pharmaceutically acceptable acid
addition salt thereof.

25 9. The compound according to claim 8
wherein R¹ is benzyl.

10. The compound according to claim 8 wherein
R² is benzyloxy.

30

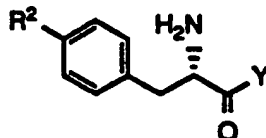
11. The compound according to claim 8 wherein
X is O.

12. The compound according to claim 11 that
35 contains three phenyl rings.

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13. The compound according to claim 12 wherein R^1 is benzyl and R^2 is benzyloxy.

14. A compound whose structure corresponds to the formula



wherein the depicted $-NH_2$ group is in the (S) configuration;

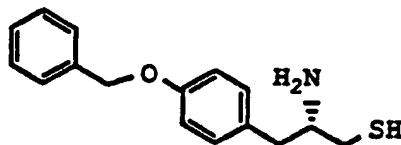
$-Y$ is selected from the group consisting of (a) phenyl, (b) trifluoromethylphenyl, (c) carboxyphenyl, (d) benzyl, (e) C_1-C_6 alkylencarboxyl, (f) C_1-C_6 alkyl, (g) C_2-C_6 alkenyl, (h) C_1-C_6 alkylenephenyl;

R^2 is hydrogen, benzyloxy or 2-naphthylmethyloxy, and a pharmaceutically acceptable acid addition salt thereof.

15. The compound according to claim 14 that contains three phenyl rings.

16. The compound according to claim 15 wherein R^2 is benzyloxy.

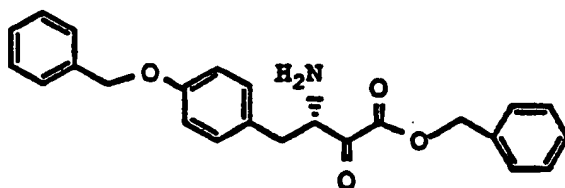
17. A compound whose structure corresponds to the formula



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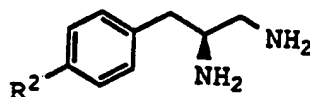
and a pharmaceutically acceptable acid addition salt thereof.

18. A compound whose structure corresponds to the formula



and a pharmaceutically acceptable acid addition salt thereof.

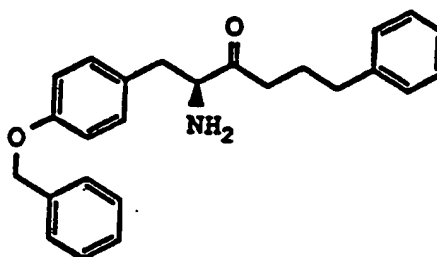
19. A compound whose structure corresponds to the formula



wherein R² is benzyloxy or 2-naphthylmethyloxy,

and a pharmaceutically acceptable acid addition salt thereof.

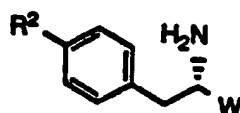
20. A compound whose structure corresponds to the formula



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and a pharmaceutically acceptable acid addition salt thereof.

21. A pharmaceutical composition comprising a leukotriene A₄ hydrolase-inhibiting amount of a compound dissolved or dispersed in a pharmaceutically acceptable diluent, said compound having a structure that corresponds to the formula



wherein the depicted -NH₂ group is in the (S) configuration;

-W is -CH₂SH, -CH₂NH₂ or C(=Z)-Y, wherein =Z is =O, or -H and -OH; and -Y is selected from the group consisting of (a) phenyl, (b) trifluoromethylphenyl, (c) carboxyphenyl, (d) benzyl, (e) C₁-C₆ alkylencarboxyl, (f) C₁-C₆ alkyl, (g) C₂-C₆ alkenyl, (h) C₁-C₆ alkylenephenyl and (i) -C(=P)-X-R¹ wherein X is O or NH and, R¹ is selected from the group consisting of C₁-C₆ alkyl, C₁-C₆ alkylencarboxyl, and benzyl, with the proviso that -Y is other than (i) when Z is -H and -OH; and

R² is hydrogen, benzyloxy or 2-naphthylmethyloxy, and a pharmaceutically acceptable acid addition salt thereof.

22. The pharmaceutical composition according to claim 21 wherein R² is benzyloxy.

23. The pharmaceutical composition according to claim 22 wherein said compound contains three phenyl rings.

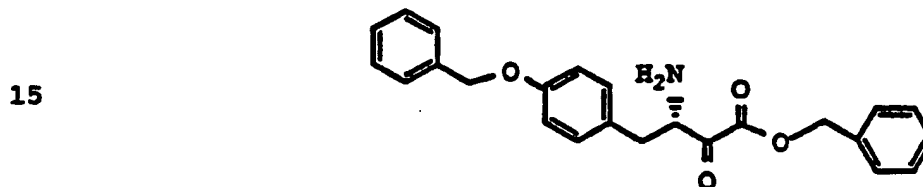
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24. The pharmaceutical composition according to claim 21 wherein -W is $-\text{CH}_2\text{SH}$ or $-\text{CH}_2\text{NH}_2$.

5 25. The pharmaceutical composition according to claim 21 wherein =Z is =O.

26. The pharmaceutical composition according to claim 25 wherein -Y is $-\text{C}(=\text{O})-\text{X}-\text{R}^1$.

10 27. The pharmaceutical composition according to claim 26 wherein said compound has a structure that corresponds to the formula



20 28. A compound whose structure corresponds to the formula



wherein the depicted $-\text{NH}_2$ group is in the (S) configuration; and

30 R^2 is hydrogen, benzyloxy or 2-naphthylmethyloxy, and a pharmaceutically acceptable salt thereof.

29. The compound according to claim 28 wherein R^2 is benzyloxy.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/03879

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : CO7C 233/08

US CL : 560/38, 39; 564/161, 170, 182, 155, 336, 306, 340

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 560/38, 39; 564/161, 170, 182, 155, 336, 306, 340

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^o	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A, 4,743,585 (Hudspeth et al.) 10 May 1988, column 83, lines 20-30	1,4,21,24
X	Chemical Abstracts, Vol. 110, issued 1989, Schwartz et al, "Aminoethanol...theri preparation", pages 23337, see abstract no. 23330m, FR,A, 2604434 J01 April 1988.	1-4,17,21,22 24
X	Biorg. Med. Chem. Lett., Vol.1, No.10, issued October 1991, Yuan et al., "Probing the inhibition...activity", see Chemical Abstract 101596t.	1,3,5,21,24
X	J. Med. Chem., Vol. 35, issued 07 February 1992, Ocain et al., "Alpha Keto Amide Inhibitors of Aminopeptidases", pages 451-456. see entire document.	1,4,21,24
X	US,A, 4,704,464 (Brunner et al.) 03 November 1987,	1,4,21,24

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

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^L document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (to be specified)	^A document member of the same patent family
^O document referring to an oral disclosure, use, exhibition or other means	
^P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

14 September 1993

Date of mailing of the international search report

25 OCT 1993

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